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JOURNAL OF DAIRY SCIENCE

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PEOPLE AND EVENTS

Professor C. E. Wylie Retires at Tennessee

C. E. WYLIE joined the faculty of the University of Tennessee in 1916. He had taught public school in Warren County, Ohio, from 1909 to 1911. He became first head of the Dairy Department in 1924 and retired August 31, 1960.

In the American Dairy Science Association, he was Vice-President and Acting Secretary in 1930 and Director in 1930–31. He has been Chairman of the following in the A.D.S.A.: Committee to Set Up Teaching Award, Breeds Relations Committee, Arrangements for Annual Meeting of the Association in Tennessee, 1951, and Official Cow Testing Section.

Professor Wylie was one of the original organizers and later Chairman of the Southern Section of A.S.D.A. In 1956, this Section gave him the Honor Award for Distinguished Service to the Dairy Industry of the South.

Professor C. E. Wylie, Head, Dairy Department, receiving citation for outstanding service to the dairy industry in the South.

He is author or co-author of 40 technical bulletins, reports, and articles, a laboratory manual, and about 65 popular magazine articles and reports.

With the assistance of the staff and others, he planned the million-dollar dairy building program, dedicated in 1949 at Knoxville, Tennessee.

Students who earned degrees in the Dairy Department are located in 25 states and three foreign countries. They hold many important positions in education, as well as in other fields.

Professor Wylie assisted with the organization of the Tennessee Jersey Cattle Club, the Tennessee Jersey Production Show, the Tennessee Dairy Products Association, the American Dairy Association of Tennessee, the Knoxville Milk Producers Association, the Chattanooga Area Milk Producers Association, and the Summer School for Town and Country Ministers at the University of Tennessee. The Annual Dairy Institute was organized by Wylie in 1953.

Professor Wylie is a member of Alpha Zeta, Phi Kappa Phi, and Gamma Alpha.

A native of Ohio, Wylie obtained his Bachelor of Science degree at Ohio State University (1915) and his Master's degree from the University of Missouri (1916). He is Chairman of the Board of Trustees of the Tennessee School of Religion and expects to give more time to this religious activity at the University of Tennessee. The Wylies live at 5809 Toole Drive, Knoxville, Tennessee. They have one daughter and three grand-daughters.

C. L. Blackman Named to Hall of Fame at Ohio State University

The portrait of an outstanding Ohio dairy leader was recently added to the Hall of Fame in the Dairy Science Department at Ohio State University. The fourteenth person to be honored is C. L. BLACKMAN, Extension Dairyman at Ohio State University.

T. S. Sutton of the College of Agriculture accepting the portrait of C. L. Blackman for the college. Mrs. Blackman was presented with a bouquet of roses.

Blackman was honored by the Ohio State University Student Branch of the American Dairy Science Association because of his outstanding contribution to the dairy industry in Ohio. At an honors banquet sponsored by the group, RUSS NYLAND, President of the

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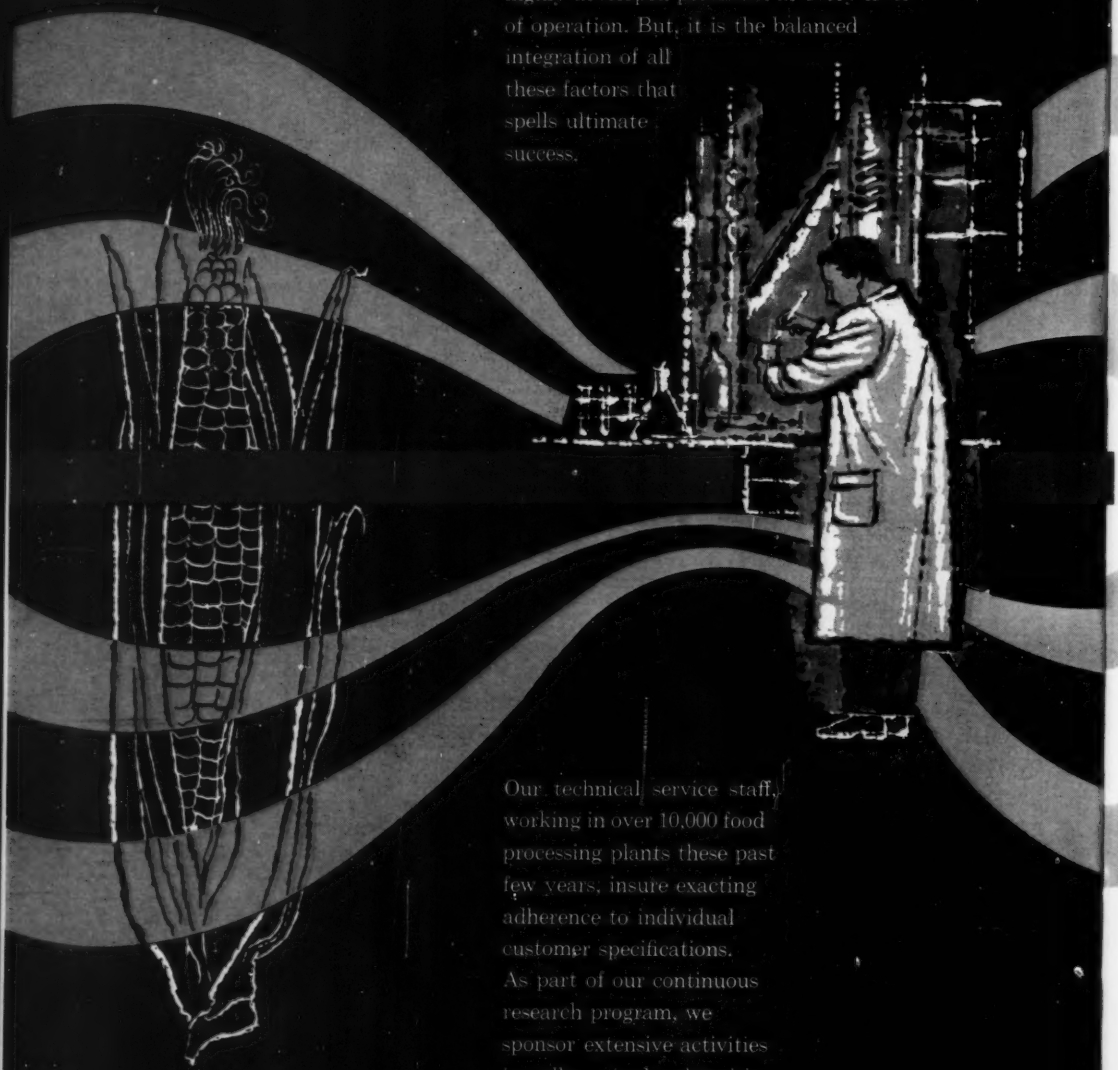

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student A.D.S.A., presented the portrait to the College of Agriculture. DEAN T. S. SUTTON accepted it for the college.

FORDYCE ELY, Chairman of the OSU Dairy Science Department, cited to banquet attendants Blackman's many accomplishments.

Blackman was born in 1893 on an island off the coast of Maine. He graduated from the University of Maine in 1916. The following year, he earned his Master's degree from Iowa State College. Following this he was a county agent in Maine. He was then for a period of 3 yr. a dairy extension specialist at Iowa State College.

Blackman then served as field secretary for the New England Holstein Association from 1921 to 1923. Following this, he was manager of Summit Farms, a Holstein farm in Maine.

In 1925 he came to Ohio as Extension Dairyman and has served in this capacity for 35 yr. Dr. Ely emphasized that Blackman has given of himself beyond the call of duty.

From January, 1947, to July, 1948, Blackman was on leave of absence to Colombia, South America, as a technician for the Office of Foreign Agriculture Relations. There he carried on extension work and taught dairy husbandry at Facultad de Agronomia de Medellin de Colombia.

For 2 yr., October 1955–57, he was on leave with the first group to go to Rajasthan, India, with T. S. Sutton of OSU.

Dr. Ely said the Blackman home is always open to those on the OSU campus from foreign lands.

"Blackman can be considered the father of artificial breeding in Ohio," said Ely, "because he started it." Ely continued, "Charles is never afraid to say he doesn't know. His rule is never compromise with the truth. He believes in keeping an open mind, a clean heart and a forgiving disposition."

He is serving his second year as secretary of the Ohio Dairyman's Association.

Other Ohio State News

B. W. THARP has joined the faculty of the Ohio State University as Assistant Professor in Dairy Technology, effective August 15. After receiving his B.S. Degree in Dairy Manufacturing from the Pennsylvania State University in 1951, Dr. Tharp joined the U.S. Navy as an officer in the Atlantic Fleet. From 1954 to 1955 he served as Assistant Plant Manager with the Tharp Ice Cream Company, Inc., Shamokin, Pennsylvania. In 1955 Dr. Tharp commenced graduate work at Pennsylvania State University and received his M.S. Degree in Dairy Science in 1957 and his Ph.D. Degree in Dairy Science in 1959. From July, 1959, he has served as Assistant Professor of Dairy Manufacturing at the University of Wyoming. At Ohio State, Dr. Tharp will be

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J. H. MARTIN has joined the faculty of the Ohio State University Research Foundation as Research Associate in the Department of Dairy Technology, effective September 1. Mr. Martin received his B.S. Degree in Dairy Manufacturing from the University of Tennessee in 1957, and his M.S. Degree in Dairy Technology from the Ohio State University in 1958. From 1958 to 1960 he has served as an Instructor in Dairy Manufacturing at Mississippi State University. While at Ohio State Martin will be working on the significance of spore-forming microorganisms in milk, on a project supported by a grant from the United States Public Health Service.

I. A. GOULD, Chairman of the Department of Dairy Technology, the Ohio State University, will be on leave from the University from August 1 to November 1. While on leave, Dr. Gould will serve as Consultant in Training and Education for UNICEF. He will work with other technical groups of the United Nations concerned with educational programs. Specifically, Dr. Gould will study educational schemes concerned with the conservation of the world milk supply. He will study the program itself, and the effectiveness of the educational programs on milk conservation programs of the various countries participating in the United Nations educational schemes, and determine how the persons are selected for these programs. As a consultant, Dr. Gould will travel in Europe and Asia, where he will be visiting dairy institutions in Belgrade, Teheran, India, Baghdad, and Beirut.

News From Illinois

K. E. EBNER of Pitt Meadows, British Columbia, Canada, recently received a grant from the National Research Council of Canada to study problems of milk secretion at the National Institute for Research in Dairying at Reading, England. Ebner will be working with DR. GUTFRIEND on this problem. The grant is for 1 yr., starting September 1, 1960.

Dr. Ebner received his Ph.D. from the Department of Dairy Science, University of Illinois, in June, 1960. The title of his thesis was Cultivation and Biochemical Characterization of Bovine Mammary Cell in Tissue Culture.

Students Awarded Scholarships at Minnesota

Six dairy industries students at the University of Minnesota have been awarded Minnesota Dairy Industry scholarships for the year 1960–61.

Receiving $300 scholarships are: T. O. AMREN, St. Hilaire; J. J. DEE, 2063-D Hoyt

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The Minnesota dairy industry provides these scholarships to aid students who plan a career in dairy industry.

News from North Carolina

W. R. MURLEY, North Carolina State College, was awarded a Danforth Scholarship to attend a summer seminar on Value Perspectives in College Teaching at Michigan State University. He was one of 23 participants who discussed such topics as Factors Involved in Changing the Values of College Students, Can Higher Education Affect Student Values, Practices in Value Oriented Courses, and Research in Critical Thinking, Attitudes and Values.

Promotions in the Department of Animal Industry, North Carolina State College, effective July 1, 1960:

Associate Professor to Professor: L. W. AURAND, W. R. MURLEY, S. B. TOVE, and L. C. ULBERG.

Assistant Professor to Associate Professor: E. U. DILLARD, LEMUEL GOODE, H. A. RAMSEY, and W. W. G. SMART.

Illinois News

H. K. WILSON has been promoted to the position of Assistant Professor of Dairy Technology at the University of Illinois. He has been research associate for 3 yr., studying the effects of high temperatures on the properties of fluid milk products. He has made significant contributions and will continue research in this area. He will also teach the course in dairy plant management, taught by DR. P. H. TRACY, Emeritus Professor of Dairy Technology, who retired on September 1, 1959, and lives in De Land, Florida.

H. K. Wilson

Dr. Wilson earned the B.S. and Ph.D. degrees at the University of Illinois and the M.S. degree at the University of California. For 23 yr. he held positions in industry as superintendent, director of laboratories, and plant manager. He is a member of Alpha Zeta, Gamma Sigma Delta, Sigma Xi, and the American Dairy Science Association.

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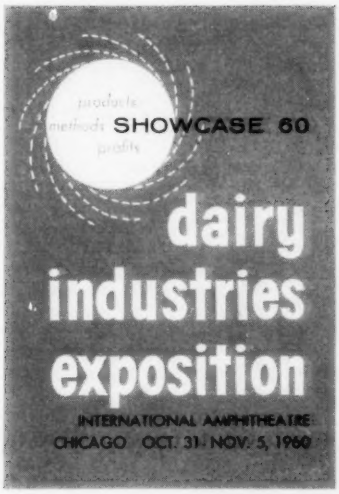

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Highest in the history of the competition, these totals were made possible by the return of an earlier $1,500 graduate research grant, unused, to DISA, enabling an increase of $500 to be added equally to the value of the three available fellowships, normally amounting to $1,800, $1,650, and $1,500.

Staged concurrently with the opening of the 22nd Biennial Dairy Industries Exposition in Chicago, Illinois, October 31, the student taste tourney is expected to attract judging teams from more than 30 colleges and universities. Winners will be announced and awards presented at a banquet to be held the next evening at the Morrison Hotel.

Judging the contest in the various divisions are N. E. FABRICIUS, Dried Milk Products Cooperative, All-Products; D. A. PETTEE, The Creamery Package Mfg. Company, Milk; ROBERT JOHNS, Kraft Foods Company, Cheese; J. H. ERB, The Borden Company, Ice Cream; B. J. ALDRICH, H. C. Christians Company, Butter.

The above officials work in conjunction with associate judges, who are: DAN ROAHEN, The Creamery Package Mfg. Company, Milk; NEIL ANGEVINE, Meyer-Blanke Company, Cheese; ROBERT LLOYD, American Maize-Products Company, Ice Cream; CHARLES WEINREICH, Cherry-Burrell Corp., Butter.

Superintendent of the Contest is L. H. BURGWALD, Marketing Specialist with the Dairy and Poultry Division of Foreign Agricultural Service, U. S. Department of Agriculture. Assistant Superintendent is D. R. STROBEL, Deputy Director, Foreign Marketing Branch, Dairy & Poultry Division, Foreign Agriculture Service, USDA.

R. H. Baum Joins Foremost Dairies

R. H. BAUM has joined Foremost Dairies, Inc., San Francisco headquarters staff, as assistant to the vice-president in charge of production and engineering.

Prior to joining Foremost Dairies, Baum was general sales manager for the Henszey Company in Waterton, Wisconsin. He is a graduate of the University of Wisconsin and holds a degree in Mechanical Engineering. Baum has also done post-graduate work at Massachusetts Institute of Technology. He will leave soon for an extended trip to Europe and Scandinavia to survey the whole milk powder market there.

Magruder Joins Cargill-Nutrena Research Staff

N. D. MAGRUDER, researcher in animal nutrition, has joined Cargill-Nutrena to head research aimed at improved feeding, housing, and management of laying hens, pullets, and replacement chicks.

Magruder began work in late July at the 840-acre Cargill-Nutrena research farm near Elk River, Minn. His activities are in cooperation with M. F. LAWATSCH, in charge of broiler and turkey research, and under direction of C. B. KNODT, research farm director.

N. D. Magruder

Magruder, a native of Martinsburg, West Virginia, holds degrees of Bachelor of Science and Master of Science from West Virginia University, and Doctor of Philosophy from Pennsylvania State University. Before joining Cargill-Nutrena he was 4 yr. on the staff of the department of nutrition at Pennsylvania State University and 3 yr. with Charles Pfizer & Sons, Inc. in Terre Haute, Indiana. He is the author of about 35 published scientific papers on nutritional research with sheep, dairy animals, swine, poultry, and various wildlife.

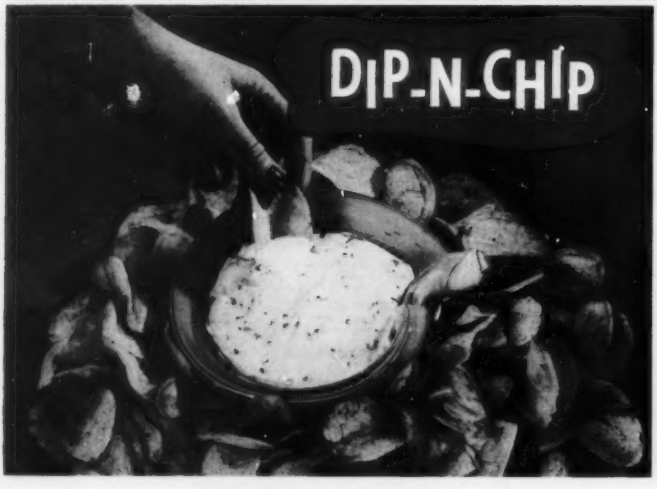
Professional groups of which Magruder is a member include Sigma Xi, American Dairy Science Association, American Society of Animal Production, American Poultry Science Association, and American Institute of Nutrition.

Daume Joins Klenzade

H. E. DAUME, JR. has been appointed Assistant Manager of Klenzade Cultured Products Division, Klenzade Products, Inc., Beloit, Wisconsin.

H. E. Daume, Jr.

Daume comes to the Company with a fine background of technical experience in dairy products manufacturing and research and development work. He is a graduate of the University of Missouri where he majored in dairy manufacturing. For the past 14 yr. since graduation he has been employed by a large nationally known dairy products corporation, where he was a Research Assistant in the Research Laboratories, specializing in culture



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3. We manufacture in small batches. Unlike other manufacturers, Kraft makes chocolate powder only in small batches. This permits more exact control over quality. A sample from each batch of Kraft's powder is made into chocolate milk and its performance tested before any of the batch is shipped to dairies.

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IOSAN® Kills Mastitis Organisms

IOSAN is a patented germicidal cleaner that kills streptococcus, pseudomonas, E. Coli, staphylococcus and other organisms that cause and spread Mastitis. Its "Tamed-Iodine" killing power has been substantiated by laboratory tests that meet hospital standards. Iosan provides safe, low cost protection when washing udders and dipping teats.

"Tattles" on milkstone. Iosan quickly cleans and sanitizes bulk tanks and other equipment. It "tattles" on hard-to-remove or overlooked accumulations of milkstone with a tell-tale yellowish-brown stain that is easy to remove. Reduces bacteria counts to consistent lows, leaves equipment sparkling clean.

Two-in-one product. Iosan saves time and labor by replacing two or more single-action products. Also reduces hot water bills because it is used in tap or lukewarm water. For a free demonstration contact your regular supplier or Lazarus Laboratories Inc., Div. West Chemical Products Inc., 42-16 West St., Long Island City 1, N.Y.

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Keep production schedules smooth

FLAV-O-LAC FLAKES

By using the Dairy Laboratories system of "Numbered Blends" you're insured complete success in every batch. Highest quality fresh culture *every week* keeps aroma, body and flavor uniform in all fermented milk products.

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work, cheese research, and trouble-shooting in the company's various plants throughout the country.

Daume also served over 3 yr. in the U.S. Army Field Artillery during World War II and holds the rank of Captain in the Army Reserve Corps.

National Dairy Council Appoints M. S. Read as Director

M. S. READ became Director of Nutrition Service for the National Dairy Council on August 15, MILTON HULT, National Dairy Council president, has announced. Dr. Read replaces DR. ZOE E. ANDERSON, former NDC Director of Nutrition Research, who resigned on June 1 to become Head of the Department of Home Economics at Wayne State University.

M. S. Read

Dr. Read comes to National Dairy Council from Virginia Polytechnic Institute, where he has since last year been Visiting Professor, Department of Biochemistry and Nutrition. From 1956 to 1959, he served as Chief of the Irradiated Food Branch, U.S. Army Medical Research and Nutrition Laboratory, at Fitzsimmons General Hospital in Denver. In this latter position, Dr. Read's work included the direction of a research team of ten scientific investigators and planning and administrative activities.

He is a graduate of Northwestern University, received his Master's and Ph.D. in biochemistry at Ohio State University. He is married, and has one daughter. Dr. Read holds numerous memberships in scientific organizations, including Sigma Xi and Phi Lambda Upsilon, the American Chemical Society, and the American Association for the Advancement of Science. He is the author of 24 scientific articles.

Completed Theses

Ph.D. Degree:

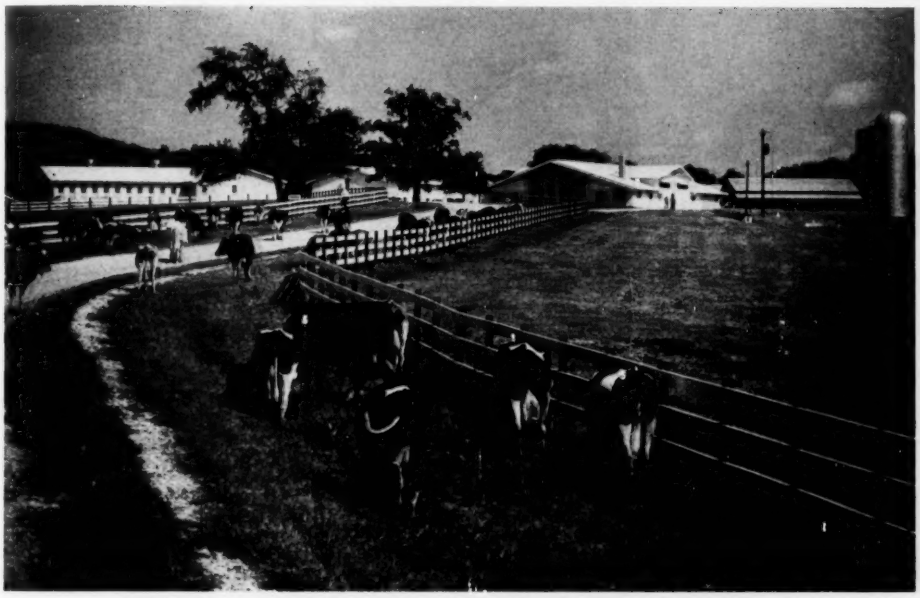
JOHN J. BETSCHER—Friction characteristics of fluid milk products. The Ohio State University, Columbus.

M.S. Degree:

HERBERT JORDAN—The digestibility of high energy rations by dairy cattle. Colorado State University, Fort Collins.

KIRVIN L. KNOX—In vitro metabolism of short-chain fatty acids in bovine rumen epithelial tissue. Colorado State University, Fort Collins.

ROBERT LINDSAY—The effect of various phys-



The Purina Farm Dairy Herd moved into this spanking new home on the Purina Research Farm at Gray Summit, Mo., this summer

What Does Purina's New Dairy Research Center Mean to the Dairy Industry?

Now in operation . . . and permitting Purina's dairy researchers to enlarge and expand many projects in the interest of better and more profitable dairying . . . is the new Purina Dairy Research Center.

Incorporating many of the most modern and up-to-date ideas used in producing milk, the Center has this one unique feature: It is designed with flexibility to facilitate the accumulation of research data from more than 300 separate grain and roughage comparisons.

This feature points to the one objective of Purina's new Center: to step up work in producing results that will be of practical value to dairymen everywhere. These results, of course, show up in the profitable results dairymen get from our Dairy Chows and Purina Dairy Programs.

The welcome mat is always out at the Purina Research Farm. If you're in the St. Louis area, drop in. We'll be happy to show you around.

MICRO-MIXED PURINA DAIRY CHOWS

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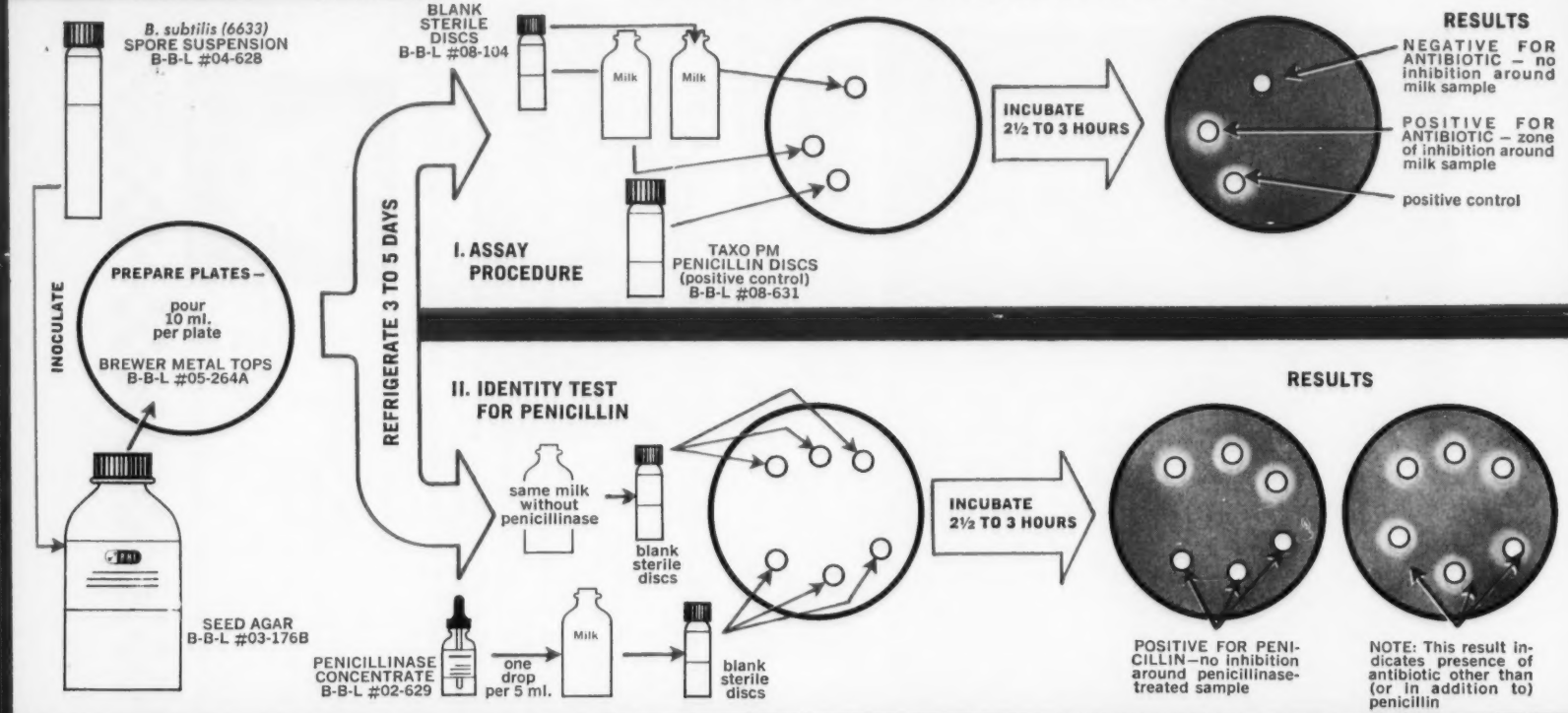
DETECTION OF PENICILLIN IN MILK



The presence of antibiotics in milk following mastitis therapy in cows has created serious public health problems and caused technical difficulties within the dairy industry. A rapid, practical laboratory procedure to assist regulatory agencies and the dairy industry in solving these problems was described by Arret and Kirshbaum.* This procedure employs rapid growth of a sensitive strain of *B. subtilis* for assaying the presence of antibiotics in milk and for determining its identity with penicillin. Inhibition of growth by the presence of as little as 0.05 unit of penicillin per ml. of milk sample is detectable within 2½ hours.

In answer to many requests for information about the availability of B-B-L products for this simplified procedure, the B-B-L Development Laboratory has prepared this TECHNICHART. It graphically illustrates the basic procedure, showing the materials necessary—**all of which are available from B-B-L.** A complete brochure with detailed technique and product listing is available upon request. No. 13

*Arret, B., and Kirshbaum, A.: J. Milk and Food Technol. **22**:329, 1959.

BALTIMORE BIOLOGICAL LABORATORY, INC.
BALTIMORE 18, MARYLAND
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ical and chemical factors on assay levels of added antibiotic in milk. Colorado State University, Fort Collins.

ROBERT O. RUPP—An evaluation of various methods of feeding Holstein steers. Colorada State University, Fort Collins.

GEORGE VIDACS—Effect of alfalfa hay and dried beet pulp rations on rumen volatile fatty acids and milk fat. Colorado State University, Fort Collins.

Symposium of Section O (Agriculture) American Association for the Advancement of Science

on

Land-Zoning in Relation to Agricultural, Suburban, Industrial, Forest, and Recreational Needs of the Future

Biltmore Hotel, New York City, December 27-30, 1960

RURAL LAND ZONING

Misuse of Land—A National Disgrace—D. A. Williams

Principles of Rural Land Zoning—E. D. Solberg

Creating Permanent Agricultural Reserves—Karl J. Belser

Tax Deferrals for Land Withheld from Development—T. W. Schulenberg

SUBURBAN PLANNING

Subdivision Control—Hugh R. Pomeroy

Soil-Survey Information for Suburban Development—Lindo J. Bartelli

New Concepts on Suburban Development Districts—Marion Clawson

Regulating Flood-Plain Development—Gilbert F. White and Robert Kates

COMMUNITY PLANNING

Small-City Community Planning—Herbert H. Smith

County and Regional Planning Board Cooperation—George H. Deming

Possibilities in Rural Planning—Sanford S. Farness

Geologic Contributions to Community Planning— William J. Wayne

FOREST AND RECREATIONAL PLANNING

Biological Requirements of Man—Paul B. Sears

Conservation of Open Spaces—Charles W. Eliot

Program Planning and Legislation for Recreation—Edward C. Crafts

Development of Water and Forest Preserves—Raleigh Barlowe

GOVERNMENT AS LAND OWNER AND REDISTRIBUTOR

Public Powers and Controls as Related to Land Use—Roscoe Martin

Law of the Open Spaces—Shirley Adelson Siegel

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Kills Mastitis Organisms. Iosan kills streptococcus, pseudomonas, E. Coli, staphylococcus and other organisms that cause and spread Mastitis. Its "Tamed-Iodine" killing power has been substantiated by laboratory tests that meet hospital standards. Iosan provides safe, low cost protection when washing udders and dipping teats. For a free demonstration contact your regular supplier or Lazarus Laboratories Inc., 42-16 West St., Long Island City 1, N. Y.

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procedure, the B·B·L Development Laboratory has prepared this TECHNICHART. It graphically illustrates the basic procedure, showing the materials employs rapid growth of a sensitive strain of *B. subtilis* for assaying the presence of antibiotics

British Town and County Planning Act—Ernest Weissman

Governmental Assistance in Area Development—Robert E. Lowry

Symposium on Recent Developments in Research Methods and Instrumentation

Final plans have been drawn for the 10th Annual Instrument Symposium and Research Equipment Exhibit to be held October 3–6 and October 4–7, respectively, at the National Institutes of Health, principal research facility of the U.S. Public Health Service. The NIH reservation is located just outside Washington, D. C., in Bethesda, Maryland.

The Symposium's opening session, presided over by SIDNEY UDENFRIEND of the National Heart Institute's Laboratory of Clinical Biochemistry, will deal with biological applications of fluorescence. Other topics on the scientific program include infrared spectrophotometry, activation analysis, ultracentrifugation and electrophoresis, microscopy, and membrane electrodes.

Instrumentation sessions will be held the mornings of October 5, 6, and 7. Manufacturers' representatives will demonstrate an electronic counter, physiological gas analyzer, fluorescent microscope, robot chemist, recording balance, and nitrogen analyzer.

Last year's equipment show attracted some 5,000 medical and technical people from federal and private research institutions, pharmaceutical houses, universities, and hospitals in all parts of the nation. Visitors also came from Asia, Latin America, the Near East, and Europe.

STUDENT CHAPTER NEWS

J. E. JOHNSTON, Editor

A Section Devoted to News of Student Members

National Student Branch Organized

June 20, 21, and 22, 1960, are days that will undoubtedly take their places in the archives of dairy history. June 20-22, the date of the 55th annual A.D.S.A. meeting, also marks the date that 22 student delegates from 16 colleges and universities throughout the nation gathered together to help make a 7-yr. dream come true.

For 7 yr. dairy personalities have devoted their time in an effort to establish a National Student Branch of the A.D.S.A. This organization became official on June 21, when the delegates voted ratification of the constitution and by-laws of the National Student Branch. The formation of this Branch had previously been approved by the executive board of the A.D.S.A. and a majority of the regular members of the Association.

The climax of the first annual meeting of the National Student Branch was the election of officers on June 22. Elected were LEWIS MEYER, Michigan State University, president; JOSEPH LINEWEAVER, Virginia Polytechnic Institute, 1st vice-president; ROBERT VAN PELT, Washington State University, 2nd vice-president; MARVIN OETTING, University of Missouri, 3rd vice-president; and FRANCIS ROTHAUGE, Oregon State College, secretary-treasurer.

The first business conducted by the executive committee was to authorize the Southern Division to proceed in its organization. This authorization allows the Southern Division of the National Student Branch to draw up a constitution and by-laws to be submitted for approval at the 2nd annual meeting of the National Student Branch, and to elect officers.

The purposes of the National Student Branch are set forth in Article II of its Constitution. "The purposes of the organization shall be:

1. To provide a channel of communication whereby information of mutual interest may be exchanged between the various member branches, and between the American Dairy Science Association—and its member branches.

2. To acquaint students with the American Dairy Science Assiciation, its scope, purpose, and program.

3. To develop leadership and promote scholarship among dairy students."

Standing committees for the next year are the constitution committee and the program committee. Committee members are Marvin Oetting, chairman, with Joseph Lineweaver and Francis Rothauge, constitution committee; and Joseph Lineweaver, chairman, with WESLEY IBBETSON and Robert Van Pelt, program committee.

In addition to the officers, student delegates of the first annual National Student Branch Meeting were D. K. NELSON, BOB LINDSAY, and CLYDE VAIR of Colorado State University; MICHAEL BENTIVEGNA, JR., Cornell University; R. W. IBBETSON, Kansas State University; J. E. BERTRAND, Louisiana State University; D. L. DINGER and J. P. HEALY, University of Minnesota; EDWARD BANDEROB, Montana State College; R. J. KLAUSING and KEITH JENKINS, Ohio State University; R. L. GILLILAND, Oklahoma State University; DUANE MATTOCK, Pennsylvania State; G. W. CLARK, South Dakota State; E. L. JENSEN and G. A. WILLARDSON,

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With other nutrients, protein is needed to repair and replace body substances used up in daily living . . . and to maintain health and vigor in all body tissues.

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The essential amino acid requirements of the normal healthy adult can be provided by 2.6 cups of milk. The minimum adult requirement for protein, when supplied by cow's milk, has been estimated by the Food and Nutrition Board as . . . 0.39 to 0.41 gm. per kilogram body weight for the adult man . . . 0.42 to 0.43 gm. per kilogram for the adult woman . . . increasing to 0.56 gm. per kilogram during pregnancy and 0.71 gm. kilogram during lactation.

Thus minimum protein and amino acid needs could be satisfied daily by . . . 3.3 cups of milk for the 70 kilogram man . . . 2.9 cups of milk for the 58 kilogram woman . . . 3.8 cups of milk for the same woman during pregnancy . . . and 4.8 cups while she nurses her baby.

Milk is man's first dietary source of protein. Cow's milk, as such and in dairy foods, can continue to supply easily digested high quality protein at all periods of life . . . in various forms . . . at any course of any meal of the day . . . in any ratio of protein to fat which may be desired.

The nutritional statements made in this advertisement have been reviewed by the Council on Foods and Nutrition of the American Medical Association and found consistent with current authoritative medical opinion.

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Utah State University; and Bob Bauer, Virginia Polytechnic Institute.

Student News Section Plans

The student news section has, in past years, failed to measure up to expected standards. There are many reasons for this among them: (1) Failure of your editor to procure adequate material and in some cases, to meet publication deadlines; (2) failure of the Student Chapters to submit news regularly and on time. During the 1960–61 year an all-out effort is to be made to develop a section worthy of the continued attention of student affiliate members. Several articles which were carried over from last spring are being published in this issue. The continuation of student chapter news is dependent on each chapter submitting news regularly. Don't wait until the end of the quarter or semester to submit a summary of activities—have your chapter reporter submit a brief article each month. Pictures are also desired, but remember to include a descriptive title explaining who is in the picture and what is going on.

Articles by leaders in the Dairy Industry are also planned, these articles to include subjects such as Job Opportunities, Trends in Dairy Science, etc. Suggestions for feature articles or methods of improving the section are solicited from all student and regular members of the Association. News should be submitted by the 5th of each month to: J. E. Johnston, Dairy Department, LSU, Baton Rouge 3, Louisiana.

Utah State University Dairy Science Club

The U. S. U. Dairy Science Club has been enjoying a varied program of activities under the direction of the Club officers; Max Sudweeks, President; Rulon Chappell, Vice President; Brad Molen, Treasurer; Ray Brog, Secretary; Dr. E. E. Starkey as advisor.

One of the most successful events was the annual cheese smorgasbord. This has become a tradition with the Club and is anticipated far in advance, not only by the students and staff but also by guests from the Dairy Industry.

At the smorgasbord a full-course dinner is served, with many varieties of cheese being featured. The cheese is displayed and markers give the names of the varieties. As part of the program, A. J. Morris spoke on the Romance of Cheese.

Scholarships were awarded during the evening. This year we were fortunate to have Daryl Chase, President of Utah State University, present the awards to the following Club members: Rulon Chappell, Weber Central Dairy Award; Eulyn Jensen, Row Ice Cream Mix Co. Award; Roy Brog, Mountain States Creameries Award; Gene Yardly, Borden Award; and Arthur Willardson, Sego Milk Co. Award.

Spring quarter events will be participation in the University Agathon program, Spring Dairy Tour, and the Annual Steak Fry.

The U. S. U. Student Branch of A.D.S.A. is looking forward to assisting with the annual meetings to be held in Logan this June.

Washington State University News

The Annual Institute of Dairying Banquet was held on March 8, 1960, at Washington State University. This year the banquet was taken over as a student function with all planning and entertainment performed by the Dairy Club members. There were 148 present, including institute delegates, faculty, and students. Jack Salvadalena was General Chairman and Tostmaster of the banquet. The main address was given by R. J. Werner on career opportunities in agriculture and dairying. Mr. Werner is the Executive Director of the Milk Industry Foundation at Washington, D.C.

The Dairy Club is also getting ready for the Annual Agri-Science Fair, which is to be held during the last part of April. Last year the club won first place with their booth.

The club is also planning to send a delegate to Logan, Utah, for the annual meeting of the American Dairy Science Association.

Dairy Club officers this year are: President, Van Youngquist; Vice-President, Dick Sherwood; Secretary, Ken Longmire; Treasurer, Bob Van Pelt; Reporter, Ron Starkenburg; representatives to the Associated Students of the College of Agriculture, Jon Hatt and Bob Zuppe.

Our advisor is Dr. U. S. Ashworth.

Wisconsin Student Activities

The Babcock Dairy Science Club concluded its yearly activities with its annual dinner meeting on the evening of May 14. A high light of the evening's events was the naming of J. A. Hardell as the winner of the Marschall Dairy Industry Award for 1960. This is the third award sponsored by the Marschall Dairy Laboratory, Inc. of Madison. It is granted annually to a resident of Wisconsin who is also an upperclassman in the University of Wisconsin, a major in dairy industry, and who has had the organized class work at the University in cheese manufacturing, and who is planning to continue his career in the dairy industry. The winner of the award is selected on the basis of these qualifications, plus his

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academic record and personal characteristics. The award was made by BRUNO STEIN, an alumnus of the University and Secretary of the Marschall Dairy Laboratory, Inc.

James A. Hardell entered the University of Wisconsin in 1950 to begin a course of study which was eventually to give him the B. S. degree with a major in Dairy Industry and the B.S. degree in Mechanical Engineering. He interrupted his University work by a 4-yr. enlistment in Naval aviation during which time he spent two 6-mo. tours of luty as plane commander on sea patrol duty in Japan, the China Sea, and near-by areas of the Far East. He has considerable practical experience in cheese factory operations with Swiss, Limburger, and other types of foreign cheese. Immediately after graduation in June he will begin his career in the Dairy Industry in the engineering department of Stoelting Brothers Company at Kiel, Wisconsin.

Hardell is married and has two children, a boy and a girl. His father, R. E. HARDELL, is well known in the cheese industry of Ohio, Wisconsin, and other states for his work as Swiss cheese expert of the United States Department of Agriculture, Bureau of Dairy Industry. He is at present working for the USDA, Agricultural Marketing Service, and is living in Monroe, Wisconsin.

Oregon State College Dairy Club News

Several graduate students and faculty members from Oregon State College plan to attend the American Dairy Science Association national meeting in June at Utah State University.

The dairy club awarded DORRIS LILLARD, graduate student, $50 to help defray expenses on traveling to Utah to present a research paper.

The club is nominating FRANCIS ROTHAUGE, 1960–'61 club president, for Secretary-Treasurer of the National Student Branch of the American Dairy Science Association.

The Club held its annual picnic May 22, at which time the graduate student award was presented along two other new awards established during the past year. Twenty-five dollars were presented to each: the outstanding senior club member and the outstanding freshman member. FRED GRIFFITH, 1959–60 club president, received the senior award and RAY MCQUEEN, who has been active in committee work and club financing activities, was presented the freshman award.

F. B. WOLBERG, who has coached the dairy cattle judging teams for many years, was presented a token of appreciation by the club and the 1959 judging team. Wolberg is retiring his coaching position this year and turning the job over to RAY KLEIWER, graduate student.

J. O. YOUNG was also presented with a gift for his service and time devoted to the club in his past 2 yr. as club advisor.

The picnic, held on a Sunday afternoon, is the last big club function of the year. All Dairy Technology and Dairy Husbandry Faculty and Staff were invited. The club paid total expenses of the affair. This year the Dairy Club used the barbecuing equipment of the Poultry Club and served a luscious dinner with barbecued chicken as the main dish. Oregon rain broke up the occasion shortly after lunch. About 65 people were present.

V.P.I. Dairy Club Holds Annual Spring Events

The Virginia Tech Chapter of American Dairy Science Association sponsored the Dairy Show and Dairy Products Judging Contest in conjunction with the annual Agricultural Exposition on May 6–7.

The Dairy Products Judging Contest was held prior to the Agricultural Exposition because of the time required to obtain the results. The contestants judged milk, ice cream, cheese, and butter. J. E. SCHAD, a sophomore in Dairy Technology, won first place in the professional group and received the Wyandotte trophy presented annually by the Wyandotte Chemicals Corporation. Second prize in the professional group went to RALPH HILL, a sophomore in Dairy Technology. He received the Cavalier cup presented annually by the Virginia Cavaliers, an association of dairy equipment manufacturers to suppliers. First place in the amateur group was W. L. MILLER, who received the small Cavalier cup.

Left to right: W. V. McClure, Manager of the 1960 Fitting and Showing Contest sponsored by the V.P.I. Dairy Science Club; W. M. Kegley, Assistant Manager of the Show; D. E. Birdsall, Grand Champion of the contest and Guernsey Breed Champion, with his prize-winning cow; C. N. Elam, Reserve Champion of the contest and Reserve Champion for the Guernsey Breed; J. R. Goodwin, Jersey Breed Champion; and Miss R. Tyler Altizer, Holstein Breed Champion.

The 1960 Dairy Show was very successfully managed by W. V. MCCLURE, with W. M. KEGLEY as assistant manager. There were 21 animals fitted, representing the Guernsey, Holstein, and Jersey breeds. There was a large

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a V.I.P. (*very important product*)
that's C.I.P. (*cleaned in place*)

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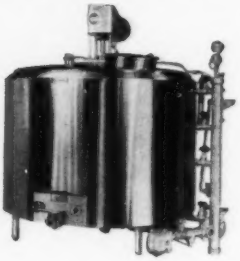

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crowd present to see MISS ANN HARRISON, Princess of the Milky Way, present ribbons to the winners. The following persons won first-place ribbons: DAVID E. BIRDSALL — Grand Champion, C. NICK ELAM, JR. — Reserve Champion, CHARLES R. PERKINS — Freshman Champion, R. TYLER ALTIZER — Holstein Champion, JOHN R. GOODWIN — Jersey Champion, DAVID E. BIRDSALL — Gurnsey Champion.

R. L. Hill, left, and W. L. Miller, second from left, waiting to receive awards for judging dairy products in the 1960 Dairy Products Judging Contest sponsored by the V.P.I. Dairy Science Club. J. E. Schad receiving the Wyandotte Trophy from Professor P. M. Large for first place in judging all dairy products, professional group.

Hill was second-place winner in judging all dairy products, professional group. Miller was first-place winner in judging all dairy products in the amateur group.

Our sincere thanks go to G. A. MILLER, fieldman for Virginia Artificial Breeding Association, for the excellent job he did as judge of the show.

Another high light in conjunction with the Dairy Show was a co-ed milking contest. The winner, MISS NANCY BARRETT, obtained 7 lb. of milk from four cows who were milked for 1 min. each.

The Dairy Club sponsored an exhibit during the Agricultural Exposition. The exhibit featured a mechanical cow with the theme, The Efficient Cow.

The Dairy Club recently elected the following new officers for 1960-61: J. A. LINEWEAVER, President; W. C. GARST, Vice-President; J. E. SCHAD, Secretary; W. G. BLOSE, Treasurer; K. O. REYNOLDS, Reporter.

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Journal of the British Society of Animal Production

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This journal is a continuation and expansion of the *Proceedings of the British Society of Animal Production* (1944-58), and has appeared in volumes of two parts but in order to make provision for the increasing flow of material submitted for publication **it will in future, commencing with Volumes 3 (1961), appear in annual volumes of three parts** in February, June and October. **The annual subscription will be 45s. ($7.50 in U.S.A. and Canada),** single parts 17s. 6d. ($2.75 in U.S.A. and Canada) net. Orders and subscriptions should be sent to:—

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STRUCTURE AND SYNTHESIS OF MILK FAT. I. GAS CHROMATOGRAPHIC ANALYSIS [1, 2]

STUART PATTON, R. D. McCARTHY, LAURA EVANS, AND T. R. LYNN
Department of Dairy Science, Pennsylvania Agricultural Experiment Station, University Park

SUMMARY

Investigation was made of the composition of milk fatty acid methyl esters (C_4-C_{18}) by means of gas chromatography employing an ionization detection system. The positions of the various methyl esters on the chromatograms were established by using reference compounds on a nonpolar (Apiezon-L) capillary column and a relatively polar (diethylene glycol succinate polyester) packed column. By this means, the presence of C_4, C_6, and C_8 through C_{18} n-alkanoic acids, the C_{16} and *cis* and *trans* C_{18} n-alkenoic acids, and the C_{18} n-alkadienoic and trienoic acids was confirmed. By selection of suitable operating conditions, it was possible to make quantitative analyses. Such analyses of milk fats collected at various seasons showed high summer levels of *trans*-octadecenoic acid, which progressively declined through fall and early winter. A comparison of summer and winter fat showed the entire C_{18} fraction to be increased, mainly at the expense of palmitate, in the summer sample. It is felt that gas chromatography can be of much value in solving problems concerning the composition of milk fat as related to its synthesis, its properties in manufactured milk products, and its nutritional value.

Since the highly illuminating work of James and Martin (6) concerning, among other things, an analysis of milk fat (goat), only a few gas chromatographic investigations of milk fat have been reported (2, 13, 14, 17). These have dealt primarily with the fatty acid composition of isolated glycerides, particularly high melting fractions. Gas chromatography has been used also in a study to reveal the positioning of fatty acids in milk fat glycerides (14). For gas chromatography to become of maximum usefulness in the analysis of milk lipids, comprehensive study of its performance in both qualitative and quantitative detection of the full range of acids occurring in milk fat will be needed. An exploration of this matter is the subject of this paper.

EXPERIMENTAL PROCEDURE

The gas chromatography instrument (Barber-Colman, Model 10) employed an ionization chamber as detector (11) and argon as the carrier gas. Both radium-226 (56 μc.) and strontium-90 (20 mc.) were evaluated as ionization sources. These two sources appeared to perform quite similarly. The strontium source yielded somewhat less background noise and, thereby, had approximately a two- to three-fold sensitivity advantage. It was used in all experiments.

Several stationary phases coated on acid-washed Celite-545, screened to 80-100 or 100-140 mesh, were studied as packings. These included diethylene glycol succinate (LAC-4-R-777, also known as -728) and adipate (LAC-2-R-446) polyesters; and Reoplex-400, another polyester of unknown constitution and

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Apiezon-L, a vacuum grease derived from petroleum. The utility of these products in gas chromatography of methyl esters has been shown (6, 8-10, 12). A criterion of satisfactory performance for such materials has been separation of the C_{18} methyl esters, particularly oleate and stearate. Although all four of the stationary phases performed well, extensive investigation was limited to diethylene glycol succinate polyester and Apiezon-L. These two phases give very good separation factors for oleate and stearate and they represent a contrast in polar-nonpolar relationship that is an aid in identification of methyl esters (9, 12).

The best columns developed to date at this laboratory for the two phases, succinate polyester and Apiezon-L, respectively, are a 9-ft. column made from heavy-wall, 1-mm. pyrex tubing containing a packing of 100-140 mesh, acid-washed Celite coated with 40 weight per cent of polyester; and a 100-ft. stainless steel capillary, 0.01-in i.d., coated with Apiezon-L. This latter column was prepared by first cleaning the tubing with benzene under 60 p.s.i. pressure and then passing through it 2 ml. of a ten weight per cent solution of Apiezon-L in benzene under 8 p.s.i. pressure. The column was conditioned for several days at 250° C. under inert gas flow. The by-pass for this column was arranged to deliver approximately 0.01 of the sample into the column. The oleate-stearate separation factor for the Apiezon-L column was 1.16 and for the polyester column, 1.18.

Reference esters were required to aid in the investigations of methyl esters from milk fat. These included methyl butyrate (Distillation Products Industries, Rochester, New York), methyl esters of the saturated, C_6 to C_{18} straight chain acids, methyl oleate, elaidate, and palmitoleate (Applied Science Laboratories, State College, Pennsylvania); and methyl esters of palmitic, oleic, linoleic, linolenic, and arachidonic acids (Hormel Foundation, Austin, Minnesota). Each of these reference esters was gas chromatographed separately on the two columns. They appeared highly pure, yielding only one peak on the chromatograms.

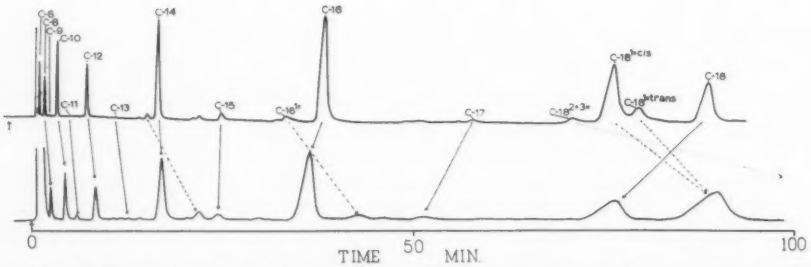

FIG. 1. Separation of methyl esters from milk fat (June) on a 100-ft. capillary coated with Apiezon-L (upper), and a 9-ft. by 1-mm. column packed with 40% diethylene glycol succinate polyester on 100-140 mesh Celite. Sample—1 μl. of dilute pentane solution of the esters. Operating conditions (upper), temperature—213° C., argon pressure—12 p.s.i., voltage—1,700, sensitivity—3×10^{-8} amp.; (lower) temperature—158° C., argon pressure—29 p.s.i., voltage—1,500, sensitivity—1×10^{-7} amp.

Milk lipids were recovered from milk by churning to butter and refining the fat to a clear oil, or by Mojonnier extraction of the total lipids. The extraction procedure recovers phospholipids (which amount to about 1% of the total lipids), while refining butter does not, and is convenient when the amount of milk available is limited. The data reported involve butter refining, except for Figures 2 and 3. The methyl esters were prepared from the fat as described

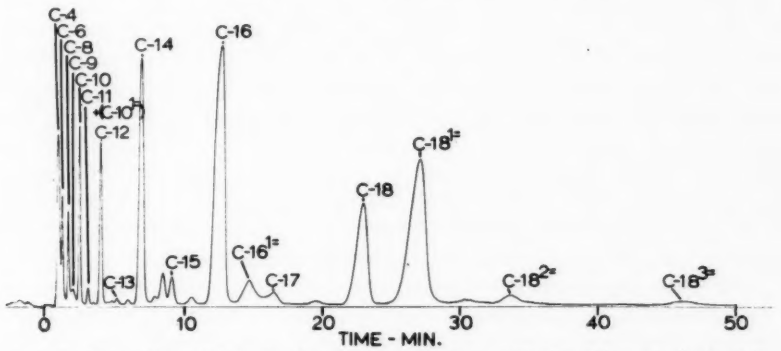

FIG. 2. Separation of 0.2 μl. of methyl esters from milk fat (November) on 9-ft. by 1-mm. column containing 40% diethylene glycol succinate polyester on 100-140 mesh Celite. Temperature—184° C., argon pressure—30 p.s.i., voltage—750, sensitivity—3×10^{-9} amp.

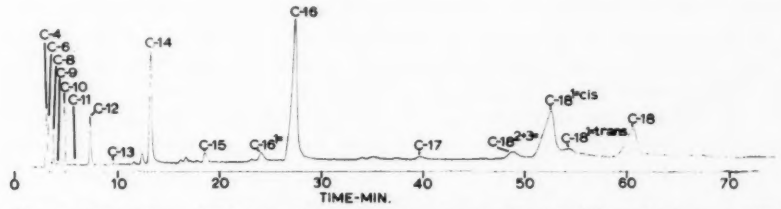

FIG. 3. Separation of 0.01 μl. of methyl esters from milk fat (November) on a 100-ft., 0.01-in. i.d. capillary coated with Apiezon-L. Temperature—213° C., argon pressure—13 p.s.i., voltage—1,700, sensitivity—3×10^{-8} amp.

by Hilditch (4). The saponification and esterification were rendered semi-micro scale by starting with approximately 0.8 g. of fat and carrying out the reactions in 6-in. test tubes capped with a small funnel and a marble to prevent loss of alcohol during gentle refluxing on a steam bath. After esterification the reaction mixture was cooled, diluted with an equal volume of water, saturated with salt, and the esters were extracted into an upper layer of 1 ml. of redistilled (35-42° C. boiling range) petroleum ether. Alternatively, the esters were recovered without solvent extraction by diluting the reaction mixture with twice its volume of water, saturating with salt, and centrifuging the esters to the surface as an oily layer. Samples for injection into the chromatography instrument were taken directly from the surface layer by means of a 10-μl. syringe.

RESULTS AND DISCUSSION

Qualitative detection. The component esters on gas chromatograms for milk fat were identified by comparing retention times with known reference compounds, using the polyester and Apiezon-L columns (for representative data, see Table 1). As observed by others (9, 12), these two stationary phases show a reversal of emergence relations between saturated and unsaturated acid esters of the same carbon chain length (Figure 1). For example, the oleate emerges from the Apiezon column before stearate, but after the stearate on the polyester column. Another aid to identification (6, 8-10, 12), used in this study, is that a plot of carbon chain lengths for a homologous series of compounds against the logarithms of retention volumes or times from a gas chromatogram yields points falling on a straight line. (The data in Table 1 can be so plotted.) This frame of reference used alone is not too reliable in application to a complex mixture of esters such as those from milk fat. The esters of acids containing odd and even numbers of carbons, together with chain branching and unsaturation, offer many possibilities of overlapping in gas chromatography.

Figure 1 shows a number of minor components from milk fat that have not yet been identified on gas chromatograms. Solution of this problem will be aided when the somewhat exotic branched chain and unsaturated acids from C_{10} through C_{17} become available as reference compounds. Use of techniques to eliminate esters of unsaturated acids from chromatograms, such as hydrogenation, bromination, or removal by lead soap fractionation (6) also will be helpful in this connection. Thus far, this investigation has revealed the following milk fat acids as methyl esters on gas chromatograms: saturated, straight chain C_4, C_6, and C_8 through C_{18}; monounsaturated C_{16} and *cis* and *trans* C_{18}; and di- and triunsaturated C_{18}. Components corresponding to 9-decenoic and linolenic acids are evident on chromatograms from the polyester column (Figure 2). These acids are known to be present in milk fat, but are obscured in analyses on the Apiezon-L column under the C_{10} saturated and C_{18} diunsaturated peaks, respectively. All of the fatty acids identified in this study are known to occur in milk fat. In fact, a substantial number of minor acids, established as present in milk fat by Shorland and others (3, 15), remains to be characterized by gas chromatography.

Column performance. At this point in the development of gas chromatography, some compromise regarding application to milk fat methyl esters will be required. The ideal analysis would involve complete resolution of every component, both major and minor, in a manner to enable quantitative determination from a single scan. This means clearly revealing C_4 to C_{26} components on the same run and in proper proportions. The problem of revealing the C_{19} to C_{26} fraction seems to be particularly vexing, in that the quantity of the whole fraction probably is not more than 2% of the total and the fraction emerges at a time in the chromatographing when detection is poor due to spreading of components. Although the ideal probably will be achieved eventually, an immediate and practical goal involves resolution of the major acid esters from C_4 through C_{18} and as many of the minor components as possible.

TABLE 1

Comparison of retention times between certain unknown methyl esters of milk fat and those for reference methyl esters of some n-alkanoic acids containing odd numbers of carbons

Reference ester	Retention time *(min.)*			
	Apiezon column[a]		Polyester column[a]	
	Unknown	Reference	Unknown	Reference
C_9	5.2	5.2	3.3	3.3
C_{11}	7.9	7.8	5.8[b]	6.1
C_{13}	13.8	13.8	11.6	12.0
C_{15}	27.8	28.2	24.4	25.0
C_{17}	60.5	61.0	51.3	51.7

[a] For details of columns, see text. Analyses were made on the Apiezon column at 213° C., and argon pressure of 13 p.s.i.; and on the polyester column at 167° C. and 30 p.s.i.
[b] This component is attributed largely to decenoate, which obscures the C_{11}.

These conditions were approximated in this study, although the quantitation of methyl butyrate remains somewhat problematical. Both succinate polyester and Apiezon-L columns have merits and limitations for the purpose. A comparison of results from the two columns (Figure 1) reveals that the Apiezon capillary gives much more refined peaks and affords a separation of the C_{18} monounsaturated ester into *cis* and *trans* components. However, under the operating conditions, which seem fairly optimum, the Apiezon capillary did not appear to separate the C_{10} and C_{12} monounsaturated acid esters from their saturated analogs, nor did it separate the C_{18} diene from the triene. It was found that methyl arachidonate would emerge from this column, under the conditions of analysis, in 135 min. Even when the sensitivity was increased threefold after emergence of the stearate, no evidence of arachidonate in the milk fat sample could be seen at the proper time. This acid ranges from 0.2 to 0.4% in milk fat (5). Although temperature or pressure programming could make the C_{19} to C_{26} components from milk fat more readily detected qualitatively, this would distort quantitative relationships, because the ionization detector becomes more sensitive as either temperature or pressure is raised (11).

Chromatograms from the polyester column revealed a slight but consistent inflection on the front side of the oleate peak (Figure 1). Methyl elaidate was observed to have a retention time 1 min. less than oleate and it is presumed that the inflection is due to the *trans* isomer. Two highly desirable qualities of the polyester column are its capacity for rapid separation and its resolution of the C_{18} series (Figure 2). The use of a 1-mm. column containing polyester

TABLE 2

Comparison between percentage composition by weight and by peak areas of a reference mixture of methyl esters chromatographed on an Apiezon-L capillary column at 213° C.

Methyl ester	Per cent		Difference
	Weight	Area	
Palmitate	25.1	25.0	−0.1
Stearate	24.7	24.7	−0.0
Oleate	25.1	25.1	−0.0
Linoleate	25.1	25.1	−0.0

TABLE 3

Comparison between percentage composition by weight and by peak areas of a reference mixture of methyl esters chromatographed on an Apiezon-L capillary column at 213° C. and at various applied voltages to the detector

Methyl ester	Per cent						
		Area			Difference		
	Weight	1400 V	1700 V	2000 V	1400 V	1700 V	2000 V
C_4	4.8	3.6	3.7	3.8	−1.2	−1.1	−1.0
C_6	4.3	4.0	3.8	4.5	−0.3	−0.5	+0.2
C_8	5.0	4.5	5.2	5.4	−0.5	+0.2	+0.4
C_{10}	5.2	5.3	4.7	5.7	+0.1	−0.5	+0.5
C_{12}	10.4	9.8	9.9	10.7	−0.6	−0.5	+0.3
C_{14}	10.0	10.8	10.1	10.1	+0.8	+0.1	+0.1
C_{16}	15.2	15.6	15.4	14.9	+0.4	+0.2	−0.3
C_{18}	14.9	16.5	16.3	14.8	+1.6	+1.4	−0.1
C_{18}^{1c}	15.2	14.9	15.4	15.1	−0.3	+0.2	−0.1
C_{18}^{2c}	15.2	14.9	15.6	14.8	−0.3	+0.4	−0.4
Total	100.2	99.9	100.1	99.8			

packing provided better over-all resolution, particularly between oleate and stearate, than did a 6-mm. column of the same length and packing. However, in routine analysis, problems of overloading and manipulation of sample size seemed to outweigh any advantage of the small-bore column, compared to a conventional 6-mm. column. The analysis on the polyester column (Figure 2) required 50 min. The same sample analyzed with the Apiezon-L capillary (Figure 3) took about 60 min. Under the conditions, these appear to be minimum times if the C_4 and C_6 components are to be separated.

The capacity of the Apiezon-L capillary to separate the *cis* and *trans* C_{18} monoenes provided some information of incidental interest. Samples of summer and winter milk fats showed a decided difference in *trans* component (Figure

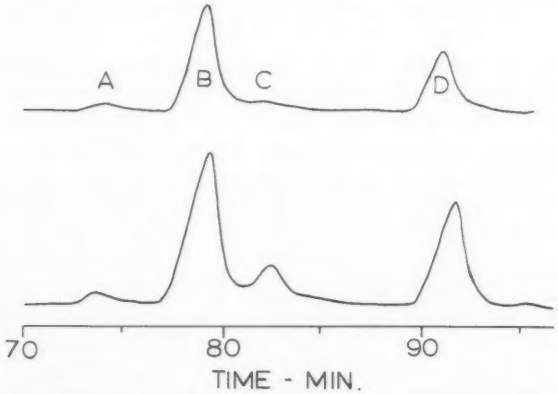

FIG. 4. Gas chromatograms showing the C_{18} methyl ester components from March (upper) and June (lower) milk fats. A—diene plus triene (linoleate plus linolenate), B—*cis* monoene (oleate), C—*trans* monoene (vaccenate and/or elaidate), D—saturated (stearate).

TABLE 4

Quantitative analysis by peak areas from gas chromatograms of fatty acid methyl esters (C_4-C_{18}) from samples of winter (March) and summer (June) milk fat

Methyl ester	Area (%)[a]		Difference (%)
	Winter	Summer	
C_4	3.5[b]	3.6[b]	+0.1
C_6	1.4	1.3	−0.1
C_8	1.1	0.9	−0.2
C_9	0.01	0.01	....
C_{10}	2.7	2.4	−0.3
C_{11}	0.05	0.03	−0.02
C_{12}	3.9	2.7	−1.2
C_{13}	0.09	0.06	−0.03
C_{14}	12.7	9.8	−2.9
C_{15}	1.0	1.1	+0.1
C_{16}	34.4	25.4	−9.0
$C_{16}^{1=}$	1.3	0.9	−0.4
C_{17}	0.7	0.7	0.0
C_{18}	11.6	15.8	+4.2
$C_{18}^{1=}$ *cis*	19.9	24.3	+4.4
$C_{18}^{1=}$ *trans*	2.5	6.4	+3.9
$C_{18}^{2+3=}$	1.5	1.9	+0.4
Unaccounted[c]	1.6	3.0	+1.4
Total	99.9	100.3	

[a] This corresponds to weight per cent.
[b] These values were calculated from butyric acid analyses by the method of Keeney (7).
[c] Peak area which could not be assigned to known components, and comprised of the same seven minor peaks from each sample.

4). A progressive decline in *trans* component from the high level of the summer was noted in analyses conducted on fat samples during the fall and early winter. The entire C_{18} fraction was materially reduced in the winter fat (Figure 4 and Table 4).

Quantitative analysis. One of the most important applications of gas chromatography in milk lipid research concerns the analysis of quantitative variations in fatty acid composition. Previous publications (8, 9, 12) have indicated that analytical results for fatty acid methyl esters are of reasonable accuracy when thermal conductivity detection is used. However, milk fat poses some unique problems because of the diverse nature of its component acids; and ionization detection involves a somewhat different group of variables than does thermal conductivity detection. As pointed out by Lovelock *et al.* (11), magnitude of response with the ionization detector is influenced by column temperature, gas flow, applied voltage, resistance connected in series with the detector, geometry of the detector cell assembly, and the amount and type of radioisotope. These workers also demonstrated that resistance of appropriate magnitude enables linear response over a fairly wide range of applied voltage.

In the quantitative calibration studies reported here, the variables in question were controlled as follows: column (Apiezon-L, 100-ft. capillary) temperature, 213° C.; argon pressure, 12 p.s.i., capillary detector cell with strontium source as supplied with the instrument; and a 2,000 megohm linearizing resistor. The scavenging gas (argon) flow through the detector was 60 ml. per minute, corresponding to 10 p.s.i. pressure. The capillary column was used because it

seemed to hold the best possibilities of sensitively differentiating and detecting all of the components of interest from milk fat. Areas of the individual peaks were computed by multiplying peak height times width at half height. The quantities of individual components were determined as percentages of total peak area.

A preliminary analysis of a reference mixture containing known amounts of palmitate, stearate, oleate, and linoleate was conducted, using an applied potential of 2,000 volts. The results (Table 2) show close agreement between composition by weight and values by analysis. To pose an analytical problem in closer keeping with the composition of milk fat, methyl esters of the short-chain acids were added to the original reference mixture and gas chromatographic analyses of this new standard were made at several applied voltages.

It is interpreted from the results (Table 3) that performance of the instrument was linear over the 1,400- to 2,000-volt range. With exception of the values for methyl butyrate, no consistent variation was observed. Of the different values, 77% varied not more than 0.5% from the composition determined by weight. The anomalous behavior of the butyrate ester was confirmed in analysis of additional reference mixtures. It invariably yielded low values. The fact that the butyrate was consistently low suggests that a correction factor for it might be used.

On the basis of the preceding calibration studies, quantitative analysis of the methyl esters from a June and early March sample of milk fat were made. The fats were from pooled herd milks. A rather decided difference in the composition of these two fats was evident (for representative data, see Table 4) due to the substantial increase in the C_{18} fraction in the summer fat. From the work of Shorland *et al.* (16), this increase seems best attributed to a combination of the factors: (1) greater availability of C_{18} dienoic acid from the lipids of pasture grass, and (2) partial hydrogenation of this acid in the rumen. One of the more notable results of this is a high level of *trans*-octadecenoic acid(s) in the summer fat. The recent investigation of Backderf and Brown (1) is pertinent to the identities and quantities of *trans* acids in milk fat.

The characteristic of capillary columns to give sharp, well-separated peaks for mixtures of compounds having great range of boiling points suggests that they will be preferred to packed columns in quantitative investigations of milk fat. The method developed in this study for quantitative analysis, employing a capillary coated with Apiezon-L, undoubtedly will bear improvement; and it is planned to evaluate longer capillaries, additional coatings, as well as to characterize further the remaining unknown minor component acids of milk fat. Nonetheless, it is felt that the method presented has value, particularly in researching variations in the fatty acid composition of milk lipids. That a rather detailed analysis can be accomplished in 1 or 2 hr., whereas it would have taken days by older, conventional methods, suggests that many problems of fat composition, especially those involving large numbers of samples, now can be undertaken. As an example, variations in milk fat composition between cows and between quarters of the udder have been investigated in a prelimi-

nary way. These variations seem to be much larger than between pooled herd samples. Although such results are not entirely unanticipated, the opportunity to study, learn from, and capitalize on such variations is at hand. The question of fat composition as a function of globule size now seems more amenable to attack. With some further improvement in the already amazing sensitivity of the Lovelock ionization detector (1×10^{-9} g. of injected sample, in our hands), analysis of a single fat globule for fatty acid composition is quite conceivable. The ionization detector, or other detector of an equal order of sensitivity, is highly desirable, in that it enables use of capillary columns and extremely small quantities of sample. These two considerations are of prime importance to lipid research.

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STRUCTURE AND SYNTHESIS OF MILK FAT. II. FATTY ACID DISTRIBUTION IN THE TRIGLYCERIDES OF MILK AND OTHER ANIMAL FATS[1, 2]

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SUMMARY

Triglyceride structure of a number of fats was studied by use of the specificity of pancreatic lipase for cleaving the fatty acids esterified on the 1 and 3 positions of the glycerol. These fats included the back, visceral, blood, and milk triglycerides of an individual cow; milk fat before and after seven days of inanition; visceral and back fat of a pig; and the depot fat from a steer.

In all these samples the per cent of C_{10}, C_{12}, and C_{14} saturated acids and the C_{11} and C_{16} monounsaturated acids, when present, were found to exist in higher concentrations in the monoglycerides resulting from the action of pancreatic lipase than in the intact fat. On the other hand, the C_{18} saturated and C_{18} di- and triunsaturated acids were found in lower concentrations in the monoglyceride derived from all fats. In pig and milk fat C_{16} fatty acid was seen to be concentrated in the 2 position, while in ruminant body fats and blood triglycerides C_{18} monounsaturated acid was evident at higher levels in the monoglyceride.

In normal milk only a slight concentration effect of C_{16} acid in the 2 position was noted, indicating almost uniform distribution. However, after seven days of inanition this acid showed a high concentration in the 2-position. A comparison, based on structure and composition of blood triglycerides and milk fat in the same animal, suggests that if blood triglycerides contribute to milk fat there either must be a rearrangement of fatty acids on the glycerol or a supplementary synthesis of triglycerides which changes the pattern of fatty acid positioning.

A useful method for determining glyceride structure is provided by the specificity of pancreatic lipase for hydrolyzing the ester linkages at the 1 and 3 positions of triglycerides (1, 8-10, 14, 15). This enzyme does not show a selectivity for the nature of the fatty acids esterified in these positions (9, 14, 15). Therefore, the fatty acid analysis of the original triglyceride, compared with the composition of the monoglyceride resulting from the action of pancreatic lipase, provides a measure of the fatty acids predominantly esterified on the 2 position of the triglyceride.

This technique was used in an extensive study by Mattson and Lutton (10). They demonstrated that, with the exception of pig fat, unsaturated acids predominate on the 2 position of triglycerides from a rather large number of animal and vegetable fats. The preferential placement of saturated acids on the 2 position of pig fat has been confirmed (13, 15). A recent report from this laboratory (11) showed that milk fat resembled pig fat in having predominantly saturated acids on the 2 position. Since milk fat showed positioning of fatty acids at variance with placement of the acids in beef fat (10), it was deduced that at least two pathways of triglyceride synthesis prevail in the

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bovine. In following this lead we analyzed the triglyceride structure of fats taken from four sources in an individual cow. These included milk, blood, visceral and back fat. In addition, milk fat, in which the fatty acid composition was modified as a result of starvation, was compared to milk from the same animal prior to withholding feed.

The analyses were based on the individual fatty acids rather than the general designation of saturated and unsaturated acids. Thus, definite trends in positioning for individual acids became evident in the various fats.

EXPERIMENTAL PROCEDURE

The four samples of fat from different locations in an individual Guernsey cow were obtained within a 2-hr. period, i.e., from last milking to slaughter. These samples included the triglycerides from milk, blood, visceral and back fat. A normal milk sample was obtained from Cow 964 before the initiation of a starvation program. At that time her daily production was 24.7 lb. of milk containing 4.1% fat. After withholding all feed for eight days her production dropped to 2.9 lb. of milk, with a fat test of 5.2%. The reported composition of milk fat produced during fasting represents the average of duplicate analyses of the A.M. milking on the sixth, seventh, and eighth day of inanition. Because of the similarity in composition the analyses have been averaged.

The pig and steer fats were obtained from the meat laboratory at the University. The previous history of these animals is not precisely known. The visceral and back fat of the pig were samples from an individual animal. The steer depot fat was obtained from an animal receiving a ration designed to produce a hard or stearic-rich fat.

All fat samples were extracted by essentially the same technique, using a 1:5:5 ethanol–ethyl ether–petroleum ether extraction solvent. To obtain a sufficient quantity of blood triglycerides, it was necessary to extract 5 liters of whole blood.

Triglycerides were isolated by silicic acid chromatography (4) before being subjected to hydrolysis by pancreatic lipase according to the procedure of Mattson and Beck (8). The resulting monoglycerides were isolated on a silicic acid column (4), their identity being confirmed by infrared spectrophotometry. After saponification the fatty acids were converted to methyl esters by refluxing 1 hr. in methanol with sulfuric acid as catalyst (3). The gas chromatographic separation of methyl esters was performed on a Barber-Colman Model 10 instrument, using a 100-ft. capillary column coated with Apiezon-L. The details of this instrumentation for separating, identifying, and quantitatively measuring fatty acid methyl esters have been reported (12). Minor component acids have not been considered in the present study nor were data taken for acids present in negligible quantities. Since the analysis of methyl butyrate under the conditions was of questionable validity, it has not been included. The type of column used does not separate C_{18} diene and triene methyl esters (linoleate from linolenate); for this reason, they have been grouped together and designated in the tables as $C_{18}^{2+3=}$.

RESULTS

A comparison of the results in this paper with those of Mattson and Lutton (10) shows close agreement when considered on the basis of the relative positioning of saturated and unsaturated fatty acids. Table 1 compares the findings for those fats common to both studies. Table 2 presents the fatty acid composition of the original triglycerides, and the monoglycerides resulting from lipase action. In all the fats C_{10}, C_{12}, C_{14}, $C_{14}^{=}$, and $C_{16}^{=}$ fatty acids, when present, were found to exist in higher concentrations in the monoglycerides. On the other hand, the C_{18} and $C_{18}^{2+3=}$ fatty acids were found in lower concen-

TABLE 1

A comparison of results from two studies for per cent of total saturated acids in the 2 position of fat triglycerides

Type of fat	Weight per cent of total saturated acids in the 2 position	
	Present study	Mattson and Lutton (10)
Beef	23	18
Cow A, back	21	....[a]
Cow A, visceral	24	....
Pig, back	65	65, 67
Pig, visceral	57	....

[a] No data.

trations in the derived monoglycerides. In pig and milk fat, palmitic acid was seen to be concentrated in the 2 position, while in ruminant body fats and blood triglycerides oleic acid was evident at higher levels in the monoglycerides. In normal milk only a slight concentration effect of palmitic acid in the 2 position was noted. However, after withholding feed for several days this acid had a much greater concentration in that position.

The percentage of each fatty acid which was on the 2 position in the original triglycerides (Table 3) can be estimated from the composition of the triglycerides and resulting monoglycerides. With the exception of palmitic acid in normal milk, none of the fatty acids approximates random distribution. A randomly distributed acid would show a concentration of roughly 33% in the 2 position. With one exception (Cow A, visceral) the level of stearic acid in the 2 position of the triglycerides was rather uniform at approximately 20%. The total stearic acid content of these fats covered a range of 9 to 25%.

All these fats show 25% or less of the C_{18} dienoic and trienoic acids in the 2 position. Regarding the placement of such acids in pig fat triglycerides, these results disagree with those of Reiser (13), who found them entirely on the 2 position. However, our results for the pig do agree with Mattson and Lutton (10), who reported an average of 22% of the linoleic and linolenic acids located on the 2 position. On the other hand, Mattson and Lutton (10) found 88% of the dienoic acid concentrated on the 2 position of beef fat, whereas our analyses show an average of 20% of the C_{18} dienes and trienes present in that position.

TABLE 2

Fatty acid composition[a] of various animal fat triglycerides and monoglycerides derived therefrom by action of pancreatic lipase

Fatty acid	C_6	C_8	C_{10}	C_{12}	C_{14}	$C_{14}^{=}$	C_{16}	$C_{16}^{=}$	C_{18}	$C_{18}^{=}$	$C_{18}^{2+3=}$
Type of fat											
Normal milk (Cow 964)											
Original triglyceride	0.9	1.2	2.4	2.8	12	....[b]	38	1.7	14	23	4.0
Monoglyceride formed	<.1	1.1	4.7	5.4	20	....	40	2.3	8.7	15	1.9
Milk, after inanition Cow (964)											
Original triglyceride	0.4	0.7	0.6	1.0	3.9	....	32	2.5	14	40	4.6
Monoglyceride formed	<.1	0.4	1.3	1.7	8.0	....	46	3.4	9.6	26	3.3
Pig, visceral											
Original triglyceride	....	....	....	....	1.8	....	26	2.8	16	42	12
Monoglyceride formed	....	....	....	....	5.0	....	60	3.5	8.6	20	2.7
Pig, back											
Original triglyceride	....	....	....	....	1.7	....	21	2.8	15	46	13
Monoglyceride formed	....	....	....	....	4.6	....	59	4.6	9.3	19	2.9
Steer, depot											
Original triglyceride	....	....	....	....	4.6	0.7	29	2.6	24	36	3.0
Monoglyceride formed	....	....	....	....	8.8	1.5	16	4.7	14	54	1.2
Cow A, back											
Original triglyceride	....	....	....	....	3.8	2.3	30	7.4	8.9	46	1.9
Monoglyceride formed	....	....	....	....	8.4	8.2	13	15	6.2	48	1.4
Cow A, visceral											
Original triglyceride	....	....	0.2	....	6.1	0.8	31	3.2	23	34	1.5
Monoglyceride formed	....	....	0.5	....	15.0	3.5	18	6.8	8.9	46	1.0
Cow A, blood											
Original triglyceride	....	....	4.5	....	4.0	1.6	26	6.4	19	34	4.2
Monoglyceride formed	....	....	7.9	....	5.9	3.4	16	10	13	43	....
Cow A, milk											
Original triglyceride	0.9	0.9	3.0	2.9	12	1.2	34	2.1	13	27	3.1
Monoglyceride formed	<.1	0.8	3.8	4.3	16	1.4	39	3.2	9.1	20	1.3

[a] As the methyl esters and expressed as percentages of total chromatogram area. These values correspond to weight per cent.

[b] Data not obtained.

DISCUSSION

Comparison of fatty acid positioning on the basis of saturated and unsaturated acids as classes is not an ideal manner of determining triglyceride structure, in that it obscures patterns in the placement of the individual fatty acids. In all the fats of this study there was a similar pattern of triglyceride structure, except in the placement of palmitic and oleic acids. It is interesting that these fats can be differentiated on the basis of saturation or unsaturation in the 2 position, mainly because of the relative positioning of these two acids. That this is true is shown by a comparison of Tables 1 and 2.

The results of this study lead us to agree with Mattson and Lutton (10), in the contention that random distribution of fatty acids does not occur in triglycerides. Randomness is not compatible with 94% of the palmitic acid occurring in the 2 position of pig fat, nor does it conform with only 20% of the stearic acid being present in the 2 position of most fats. There appears to be at least a partially directed esterification for each individual fatty acid. This is true when the results are considered either on a molar per cent or a weight per cent basis. The determining factor for this direction is not clear,

TABLE 3

Proportions of individual fatty acids located on the 2 position in a number of fat triglycerides expressed as percentages of the total content of the individual acids in the fats[a]

Fatty acid	C_6	C_8	C_{10}	C_{12}	C_{14}	$C_{14}{}^{=}$	C_{16}	$C_{16}{}^{=}$	C_{18}	$C_{18}{}^{=}$	$C_{18}{}^{2+3=}$
Type of fat											
Normal milk (Cow 964)	....[b]	31	65	64	56	....	34	45	21	22	16
Milk after inanition (Cow 964)	....	19	72	57	68	....	48	45	23	22	24
Pig, visceral	....	....	....	....	93	....	77	42	18	16	8
Pig, back	....	....	....	....	90	....	94	55	21	14	7
Steer, depot	....	....	....	....	64	71	18	60	19	50	13
Cow A, back	....	....	....	....	74	100	14	68	23	35	25
Cow A, visceral	....	....	83	....	82	100	19	71	13	45	22
Cow A, blood	....	....	59	....	49	71	21	52	23	42	....
Cow A, milk	....	30	42	49	44	39	38	51	23	25	14

[a] Calc.: $\frac{M}{3T} \times 100 =$ per cent of acid in 2 position, where M is the per cent concentration of the acid in the monoglycerides and T is the per cent concentration of that acid in the triglycerides.

[b] Data not obtained.

in that it apparently does not depend simply on chain length or degree of unsaturation.

A comparison of palmitate distribution in normal milk fat, with the distribution in fat produced during inanition, raises the possibility that two mechanisms for triglyceride synthesis are operative in milk fat synthesis. In normal milk the concentration of palmitate in the 2 position was only slightly greater than the level of random distribution. However, after withholding feed from the cow for several days the concentration of palmitic acid in the 2 position became marked. This could indicate that whatever process places palmitate on the 2 position was not as greatly inhibited by starvation as the process which places this acid on the terminal positions. Of course, all mechanisms of milk fat synthesis were reduced during fasting, as shown by the substantial decrease in fat yield.

Although the fatty acids of blood triglycerides have been shown to contribute to milk fat synthesis (2), the quantitative contribution of this source is not known. From the present results it is apparent that if blood triglycerides contribute to milk fat, the fatty acids must either be rearranged on the glycerol molecule or there must be a supplementary synthesis of milk fat which tends to compensate by concentrating palmitate in the 2 position. The data for fatty acid composition of blood triglycerides from this study are essentially similar to those given in previous reports (6, 7).

A comparison of fatty acid composition of blood and milk triglycerides provides a basis for interesting speculation. If it is assumed that all the oleate of milk fat comes from blood triglycerides, then the ratio of other acids to oleate in the blood triglycerides, compared with the same ratios in milk fat, indicates the blood triglycerides could provide all the oleate, linoleate, stearate, palmitoleate, myristoleate, and 50% of the palmitate plus 24% of the myristate in milk fat. In addition, the blood triglycerides could provide all the C_{10} fatty acid necessary for milk fat. Only in blood and milk triglycerides was this great

a concentration of C_{10} acid detected. However, in analysis of blood triglycerides from another cow, not reported here, the C_8, C_{10}, and C_{12} fatty acids accounted for 3% of the total fatty acids. The presence of short chain acids in blood may have significance in relation to the recent report of James and Lovelock (5), on the fatty acid synthesizing ability of whole blood.

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CHEDDAR CHEESE FLAVOR. II. CHANGES IN FLAVOR QUALITY AND RIPENING PRODUCTS OF COMMERCIAL CHEDDAR CHEESE DURING CONTROLLED CURING [1]

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SUMMARY

Fourteen lots of Cheddar Cheese were obtained immediately following manufacture from commercial factories selected at random in New York, Wisconsin, Oregon, and Ohio. The cheese was cured for 2 mo. at 50° F., followed by curing at 40° F. for 10 mo. The following analyses were performed periodically: grading, evaluation for characteristic flavor, ammonia, hydrogen sulfide, free fatty acids, free amino acids, acidic and neutral carbonyl compounds, pH, and total bacterial count.

The initial quality of the cheese ranged from U.S. Grade A to Below Grade, with most of the cheese showing variation in quality during the 12-mo. curing period. In general, concentrations of ammonia, free amino acids, and free fatty acids increased continuously during curing, whereas hydrogen sulfide and total bacterial counts fluctuated. Raw milk cheese contained higher concentrations of hydrogen sulfide than pasteurized milk cheese, with no conclusive difference being apparent in ammonia, free amino acids, and free fatty acids.

Statistical analysis of the results indicated significant correlations at the 5 and 1% level between characteristic Cheddar Cheese flavor scores and pH, ammonia, and hydrogen sulfide after 3 mo. of curing.

The flavor of the Cheddar Cheese appeared to be related more to the ratio of free fatty acids and hydrogen sulfide concentrations than to any other compounds or combinations of the compounds included in this study. The merits of relating characteristic Cheddar flavor to free fatty acids and hydrogen sulfide, and the possibility of using these compounds as an index of Cheddar Cheese flavor, are discussed.

In the previous paper (8) results were presented of the chemical analysis and flavor evaluation of commercial Cheddar Cheese which had been selected at random without regard to age, quality, type of milk, or manufacturing plant. General observations were made in respect to the presence and concentration of various constituents produced during ripening of the cheese, and their possible relationship to flavor quality and grade.

In the second phase of the study, selected commercial Cheddar Cheese was obtained immediately after manufacture and was then subjected to a study of chemical and flavor changes under controlled curing conditions. Results are presented in this paper.

EXPERIMENTAL PROCEDURE

Fourteen lots of Cheddar Cheese, manufactured during late June or early July, were obtained immediately following manufacture from 11 different plants in Wisconsin, New York, Oregon, and Ohio. A Wisconsin plant supplied two and a New York plant three lots of cheeses. Information was furnished by the plants regarding the milk supply and manufacturing methods (Table 1).

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TABLE 1
Certain manufacturing characteristics and ripening changes of commercial Cheddar Cheese

Cheese No.[a]	Milk		Cheese				
	Quality[b]	Treatment	Milling acidity	Moisture (%)	Salt (%)	pH 0.5 mo.	pH 12 mo.
1	A	Raw	0.61	36.4	1.5	5.0	5.3
2	A	Raw	0.61	36.6	1.7	5.1	5.2
3	A	Raw	0.58	37.1	1.7	5.1	5.4
4	A	Raw	0.68	37.1	1.5	5.3	5.4
5	A	Raw	0.65	33.9	1.8	5.2	5.5
6	B	Heated[c]	0.49	37.2	1.5	5.0	5.4
7	A	161° F.-17 sec.	0.47	37.7	1.5	5.0	5.4
8	A-B	Pasteurized	0.45	40.2	1.5	5.2	5.5
9	B	165° F.-16 sec.	0.51	38.0	1.4	5.1	5.7
10	B	161° F.-17 sec.	0.45	37.2	1.6	5.4	5.7
11	B	170° F.-20 sec.	0.50	41.8	1.4	5.1	5.6
12	B	165° F.-20 sec.	0.45	36.4	1.4	5.0	5.5
13	B	163° F.-16 sec.	0.41	34.4	1.5	5.3	5.6
14	A	Pasteurized	0.45	33.5	1.7	5.4	5.6

[a] Cheeses 1, 2, 3, and 4 manufactured in New York; 6, 7, 8, and 10 in Wisconsin; 9, 11, 12, and 13 in Ohio; 14 in Oregon.
[b] Quality determined by bacterial count or reduction test.
[c] Heated at 145° F. for 17 sec.

Six lots of cheese were made from raw or phosphatase-positive milk and eight from pasteurized milk. Each lot of cheese consisted of from three to six units, the number depending on the weight of a unit: (23 lb. or less—six units per lot; 40 lb. and above—three units per lot). The cheese was cured for 2 mo. at 50° F., followed by curing at 40° F. for 10 mo.

Analyses for ammonia, hydrogen sulfide, free fatty acids, free amino acids, acidic and neutral carbonyl compounds, pH, and total bacterial counts were conducted after 0.5, 1.5, 3, 6, 9, and 12 mo. of curing. In addition, moisture and salt determinations were made at the 0.5-mo. examination. The methods employed, except for the bacteriological analysis, were reported previously (8). Results for the carbonyl compounds have been presented (7).

Samples (1 g.) for bacterial determinations were obtained aseptically from different sections of the cheese by selecting portions of plugs removed with a cheese trier. The cheese was macerated in 9 ml. of 2% sterile sodium citrate solution and plated in standard Plate Count Medium. The plates were incubated at 32° C. for three to four days.

Two official graders from the USDA Inspection and Grading Branch evaluated the cheese according to the U.S. Standards after 0.5, 3, 6, 9, and 12 mo. of curing. In addition, the graders evaluated the cheese for intensity of characteristic flavor content.

RESULTS

Moisture, salt, and pH. The moisture and salt contents of the cheese ranged from 33.5 to 41.8% and from 1.4 to 1.8%, respectively (Table 1). With the exception of two cheeses (8, 11) the moisture contents were within the range normally encountered in Cheddar Cheese.

The pH values of the cheese ranged from 5.0 to 5.4 and 5.2 to 5.7 after 0.5

and 12 mo. of curing, respectively (Table 1). The pH for all of the cheese increased with curing, although the rates of increase differed somewhat for the individual cheeses. The pH values of raw milk cheese (one heated) were generally lower than those of pasteurized milk cheeses, the pH ranges after 12 mo. of curing being 5.2 to 5.5 and 5.4 to 5.7, respectively.

Organoleptic evaluation. Quality evaluations are presented in Table 2. Initially, three cheeses were of A grade, four of B grade, six of C grade, and one

TABLE 2

Grades and characteristic flavor values (C.F.) of commercial Cheddar Cheese during curing

	Age in months									
	0.5		3		6		9		12	
Cheese No.	U.S. Grade	C.F.[a]	U.S. Grade	C.F.[a]	U.S. Grade	C.F.[a]	U.S. Grade	C.F.[a]	U.S. Grade	C.F.[a]
1	C	2	A	6	B.G.[b]	4	A	5	B	5
2	B	3	B	4	B.G.[b]	4	B	4	B	4
3	C	0	C	4	C	4	C	5	C	5
4	B+	1	A−	6	A−	5	C+	4	B	6
5	A	3	C	1	A	5	B	4	A	6
6	A	2	B	3	A	4	A	4	A	5
7	A	1	B	1	A−	3	A	4	B+	4
8	B	0	A−	3	B	5	A	5	A	5
9	C	1	A	5	A	6	A	6	B+	5
10	C	0	B	2	B.G.[b]	4	B	3	B	4
11	C	0	C	1	C	3	C	3	C	2
12	C	0	C	1	B+	5	C	3	B−	3
13	B.G.[b]	0	C	1	C	3	C	3	C	3
14	B	1	C	1	B	1	C	1	C	1
Average		1.0		2.8		4.0		3.9		4.2

[a] C.F.—0–none; 2–very slight; 4–slight; 6–definite; 8–pronounced.
[b] B.G.—Below grade.

Below Grade. During curing the grade of most of the cheeses changed. One cheese (1) varied over the entire grade scale and several cheeses changed as much as three grades. Two cheeses (3 and 11) were graded C initially because of a major texture defect ("pinny"), and this grade persisted throughout the storage period.

Acid and unclean were the predominant flavor defects at all stages of curing. The unclean flavor developed into a sulfide defect in two cheeses (6 and 7) after 9 to 12 mo. of curing. Bitter was a common flavor criticism after 6 mo. of curing. The lots of cheese consistently criticized for acid, unclean, and/or bitter (1, 2, 3, 4, 5, 6, 7, 9) received better grades and/or characteristic flavor values than cheeses with other defects.

The cheese varied widely in the intensity of the characteristic Cheddar Cheese flavor at each of the evaluations. If arbitrary minimum acceptable standards for this characteristic were established as 3 for 3-mo.-old cheese and 5 for cheese cured 6 mo. or longer, only about one-half of the lots could be classed as satisfactory even after 12 mo. of curing.

The major average increase in the intensity of characteristic Cheddar flavor occurred between 0.5 and 3 mo. for one-half of the cheeses and between

3 and 6 mo. of curing for the other half, with little further change apparent after 6 mo. Raw milk cheese was predominant in the former group and pasteurized milk cheese in the latter. Exceptions to the general trend were apparent. Cheese 5 registered characteristic flavor intensity values of 3-1-5-4-6 at the 0.5, 3, 6, 9, and 12 mo. periods, respectively, and Cheese 14 did not develop characteristic flavor between the 0.5 and 12 mo. periods.

Ammonia. Concentrations of ammonia (Table 3) ranged from 4.7 to 16.4 m*M*/g in 0.5-mo. cheese and increased to values ranging from 26.5 to 74.9 m*M*/g by the 12-mo. period. The average increases for raw and pasteurized milk cheese were not greatly different, indicating that the breakdown of product to ammonia may proceed at about equal rate in raw and pasteurized milk cheese manufactured under different conditions. Differences in the rates of appearance and the actual concentrations of ammonia were greater between individual lots within the two groups than between the averages of the two groups.

An analysis of the data does not reveal any definite relationship between intensity of characteristic flavor and concentration of ammonia. For example, in the case of the five cheeses made from raw milk the ammonia concentrations were remarkably uniform after 3 mo. (28.1 to 29.0 m*M*/g); whereas, the characteristic flavor values varied widely (from 1 to 6). After 12 mo. Lot 1, which showed the greatest increase in ammonia (70.2 m*M*/g), had decreased slightly in characteristic flavor value from 6 to 5. Lots 2 and 5 exhibited similar increases in ammonia at 12 mo. (50 and 45 μg/g), but the characteristic flavor development in the two cheeses differed distinctly: Lot 2 did not change from its 3rd-mo. value of 4, whereas Lot 5 increased from 1 to 6. The pasteurized milk cheese exhibited similar lack of relationship between ammonia production and flavor development. As an example, Cheese 10 contained a much higher concentration of ammonia generally than did Cheese 9 throughout curing, but the latter was definitely superior in flavor. On the other hand, Cheese 8 at 12 mo. was equivalent to Cheese 9 in flavor, but possessed more than twice as much ammonia. These results all tend to indicate that ammonia per se may not contribute appreciably to characteristic Cheddar Cheese flavor.

Hydrogen sulfide. Results for the hydrogen sulfide analysis (Table 3) revealed values ranging from 0.7 to 3.4 m*M*/100 g. in the 0.5-mo.-old cheeses and from 1.0 to 2.9 m*M*/g in the 12-mo.-old cheese. There was no consistent change in the hydrogen sulfide concentrations with curing, either for the individual cheeses or for the average values of the raw and pasteurized milk lots. In general, the values tended to be highest at the 1.5-mo. period and to have a second high peak after 6 mo. of curing.

For the raw milk cheeses, the average values increased from 2.3 to 3.7 m*M*/100 g. between the 0.5- and 1.5-mo. periods and were 2.3 at the end of 12 mo. The pasteurized milk cheese revealed the same trend, but the hydrogen sulfide concentrations of the lots in this group were lower than those for the raw milk lots: 1.2 and 1.6 m*M*/100 g. after 0.5 and 12 mo. of curing, respectively. The greatest differences in hydrogen sulfide concentrations between raw and pasteurized milk cheese occurred through the first 6 mo. of curing. Also, the

TABLE 3

Variations in concentrations of ammonia and hydrogen sulfide in commercial Cheddar Cheese during curing

Compound	Milk and No. of lots	Age in months											
		0.5		1.5		3		6		9		12	
		Average[a] concentration	γ[b]	Average[a] concentration	γ[b]	Average[a] concentration	γ[b]	Average[a] concentration	γ[b]	Average[a] concentration	γ[b]	Average[a] concentration	γ[b]
Ammonia	Raw[c] (6)	7.9	2.261	10.9	1.414	27.0	3.676	30.3	5.657	40.6	7.836	49.0	16.884
	Pasteurized (8)	8.8	3.564	8.8	2.583	22.8	6.473	28.5	7.436	35.1	13.041	44.9	17.467
Hydrogen sulfide	Raw[c] (6)	2.3	0.779	3.7	1.104	2.4	0.618	3.2	0.710	2.4	0.045	2.3	0.550
	Pasteurized (8)	1.2	0.396	2.0	0.155	1.5	0.562	1.8	0.617	1.6	0.164	1.6	0.568

[a] Average concentration: Ammonia—micromoles per 1 g. of cheese. Hydrogen sulfide—micromoles per 100 g. of cheese.

[b] Standard deviation.

[c] One lot of cheese made from milk heated at 145° F. for 17 sec.

fluctuations in hydrogen sulfide concentrations, which occurred after 1.5 mo., were considerably greater in raw than in pasteurized milk cheese.

Considering the raw and pasteurized milk cheeses as groups, a relationship appears to exist between hydrogen sulfide concentrations and characteristic Cheddar flavor values. The raw milk cheese, which contained the highest average concentrations of hydrogen sulfide, exhibited the highest average characteristic flavor values at all periods (1.8 and 0.4 for raw and pasteurized milk groups, respectively, after 0.5 mo., and 5.2 and 3.4 after 12 mo.).

Another indication of a relationship between hydrogen sulfide and characteristic Cheddar flavor was illustrated with Lot 8. Although made from pasteurized milk, this cheese was considered by the judges to have a flavor similar to that of raw or heated milk cheese from the 6-mo. period and on. The hydrogen sulfide content of this cheese at 6 mo. equalled that of the raw milk group of cheeses (3.2 m*M*/100 g.).

Individual exceptions to the over-all trend in the hydrogen sulfide–characteristic Cheddar flavor relationship were apparent, particularly in the pasteurized milk group of cheeses. For example, Lot 9, which rated among the better cheeses in over-all characteristic Cheddar flavor value, was below average with respect to hydrogen sulfide values. Lot 14, the poorest lot in the entire study with respect to characteristic Cheddar flavor, contained considerably higher concentrations of hydrogen sulfide than most pasteurized milk lots. These observations would indicate that compounds other than hydrogen sulfide are a part of characteristic Cheddar flavor.

Two cheeses are of special interest: Lot 6 at 12 mo. and Lot 7 at 9 and 12 mo. At these periods, the two lots were criticized for a sulfide flavor. Although the two lots contained relatively high concentrations of hydrogen sulfide (2.4 and 2.5 m*M*/100 g., respectively, at 12 mo.), the amount was much less than in other cheeses which did not possess the sulfide defect. This indicated that the hydrogen sulfide concentration alone is not the cause for this particular flavor problem.

Free amino acids. The cheese was analyzed for 12 different amino acids at each of the evaluation periods. The results revealed wide ranges in the concentrations of these amino acids in the different lots at specific periods. There was no evidence of any consistent relationship between the amino acids and the quality of the cheese as a whole. However, as a basis for comparison, amino acid results are presented for six lots of cheese varying in characteristic Cheddar flavor values at 12 mo. of curing (Table 4). Of the 12 amino acids, glutamic acid, threonine, leucines, and glycine were present in the greatest concentrations in most of the lots (average values of 21.8, 17.8, 17.3, and 16.5 m*M*/g, respectively). In contrast, serine was present in the lowest concentrations (0 to 2.2 m*M*/g).

Although a general lack of correlation is apparent between individual free amino acids and characteristic Cheddar flavor values, it appears that a relationship may exist between the intensity of the flavor and the total concentration of free amino acids. For example, with the characteristic Cheddar flavor values

TABLE 4
Amino acid concentration in selected lots of commercial Cheddar Cheese after 12 mo. of curing

	Characteristic Cheddar Cheese flavor value and grade						
	6 (B)	5 (B+)	4 (B)	3 (B−)	2 (C)	1 (C)	Average
	Lot 4	Lot 9	Lot 2	Lot 12	Lot 11	Lot 14	
Amino Acid	Micromoles of amino acid per gram of cheese						
Aspartic Acid	10.9	11.0	7.7	4.4	4.4	13.2	8.6
Glutamic Acid	19.8	26.4	26.4	20.9	19.8	17.6	21.8
Serine	2.2	0.1	2.2	0.1	0.1	0	0.8
Glycine	20.9	13.2	18.7	19.8	8.8	17.6	16.5
Threonine	20.9	24.2	13.2	17.6	11.0	19.8	17.8
Alanine	12.5	10.9	9.2	9.2	5.0	12.5	9.9
Arginine	5.0	8.4	11.7	8.4	10.0	10.0	8.9
Tyrosine	6.7	5.0	8.4	5.0	3.3	7.5	6.0
Valine	8.4	13.4	11.7	9.2	7.5	15.9	11.0
Methionine	8.4	6.7	7.5	5.0	3.3	4.2	5.9
Phenylalanine	15.9	13.4	10.0	10.9	8.4	9.2	11.3
Leucines	21.7	16.7	16.7	14.2	13.4	20.0	17.3
Total	153.3	149.4	143.4	124.7	95.0	148.4	135.8

varying from 6 to 2 (Lots 4, 9, 2, 12, and 11) the concentrations of total free amino acids decreased from 153.3 to 95.0 m*M*/g. Lot 14 was an exception to this relationship, since it possessed a flavor value of 1 but had a total free amino acid content which equalled that of the cheese with a characteristic flavor value of 5 (148.4 m*M*/g). As indicated, the hydrogen sulfide concentrations in Lot 14 were also much higher than would be expected of a cheese with its low intensity of characteristic flavor. Lots 1 and 14 compared quite well with respect to concentrations of protein degradation products (ammonia, hydrogen sulfide, and free amino acids). If Lot 1 can be considered a normal lot of Cheddar Cheese, as may be assumed from its flavor quality value (5 at 12 mo.), it is apparent that the cause of the poor flavor quality of Lot 14 must be found in the breakdown of products other than protein.

Free fatty acids. Table 5 presents results for free acetic, propionic, butyric, and other fatty acids (those with carbon chain length greater than four) for the 0.5, 6, and 12 mo. periods. Acetic acid was present in the greatest concentrations and ranged from 2.3 to 17.4 m*M*/g after 0.5 mo. and from 6.1 to 26.1 m*M*/g after 12 mo. of curing. The concentrations of acetic acid increased during curing for all lots except Lot 14. The majority of the cheeses had values ranging from 10 to 20 m*M*/g during the period. However, Lots 5, 6, and 7 yielded comparatively low results for all periods.

The propionic acid and butyric acid concentrations were of the same magnitude, ranging from 0.1 to 0.6 m*M*/g after 0.5 mo. and from 0.4 to 1.9 m*M*/g after 12 mo. of curing. Both acids tended to increase in concentration with curing, but somewhat inconsistently.

The concentrations of the other fatty acids ranged from 2.1 to 7.3 m*M*/g after 0.5 mo. of curing. Generally, the values increased appreciably with curing (exception Lot 14) and the concentrations after 12 mo. of curing were from 3.8 to 5.5 m*M*/g.

TABLE 5

Concentrations of free fatty acids in commercial Cheddar Cheese during curing

Cheese No.	Milk	Age in months	Micromoles of fatty acid per 1 g. of cheese			
			Acetic	Propionic	Butyric	Others[a]
1	Raw	0.5	11.4	0.1	0.3	4.3
		6	18.9	0.4	1.0	7.5
		12	19.3	1.3	1.0	8.9
2	Raw	0.5	10.5	0.1	0.3	3.8
		6	17.8	0.4	0.7	5.4
		12	16.4	1.6	0.9	7.5
3	Raw	0.5	13.0	0.1	0.1	4.5
		6	16.2	0.4	0.9	5.6
		12	18.9	0.4	0.9	6.8
4	Raw	0.5	12.1	0.1	0.6	7.3
		6	20.5	0.7	1.4	10.0
		12	22.8	1.5	0.7	12.0
5	Raw	0.5	6.1	0.6	0.1	4.2
		6	9.5	1.6	0.7	6.6
		12	14.5	0.8	1.7	9.9
6	Heated[b]	0.5	2.3	0.1	0.2	2.1
		6	3.2	0.7	0.5	4.5
		12	7.8	0.6	0.6	5.3
7	Pasteurized	0.5	2.9	0.1	0.2	2.4
		6	2.9	0.2	0.8	3.4
		12	6.1	0.7	0.6	4.8
8	Pasteurized	0.5	8.6	0.1	0.2	2.1
		6	16.7	0.6	0.3	4.5
		12	18.7	1.6	0.8	5.1
9	Pasteurized	0.5	16.0	0.1	0.3	2.5
		6	20.2	0.4	1.1	9.7
		12	23.5	1.6	0.7	11.0
10	Pasteurized	0.5	10.2	0.1	0.5	4.2
		6	19.6	1.1	1.7	9.8
		12	19.6	1.6	1.1	13.7
11	Pasteurized	0.5	10.4	0.3	0.3	4.2
		6	15.0	0.6	0.9	7.7
		12	16.8	1.3	0.8	10.8
12	Pasteurized	0.5	17.4	0.2	0.5	5.1
		6	24.2	0.5	1.4	8.6
		12	26.1	0.9	1.2	10.9
13	Pasteurized	0.5	14.1	0.2	0.3	4.0
		6	19.6	0.9	1.5	10.6
		12	21.4	1.4	1.3	15.5
14	Pasteurized	0.5	13.4	0.2	0.3	3.4
		6	11.5	0.2	0.5	2.5
		12	10.1	1.9	0.7	3.8

[a] Others—Free fatty acids with carbon chain lengths greater than four.

[b] Heated at 145° F. for 17 sec.

There was no clear-cut evidence that the concentrations of free fatty acids were related to either the type of milk (raw or pasteurized) or to the characteristic flavor values of the cheese. However, an indication of the significance of free fatty acid formation to characteristic flavor development in Cheddar Cheese during curing is illustrated by Lot 14. This cheese had no appreciable characteristic flavor, apparently normal protein degradation, but no increase in the acetic acid and the other fatty acids during curing. Therefore, this

would tend to implicate these acids as being involved, either directly or indirectly, in the failure of this cheese to develop characteristic Cheddar flavor.

Bacteria. Considerable variation existed in the total number of bacteria observed in the 14 lots of cheese. The bacterial counts in raw milk cheese ranged from 2 to 170 million/g at the 0.5-mo. period and from 3.0 to 35 million/g at the 12-mo. period. The corresponding figures for the pasteurized milk cheese were 0.2 to 350 million/g and 0.9 to 60 million/g.

Relationship of grade and characteristic flavor intensity to ripening products. Statistical analyses were made to determine the possible relationship between the quality of the cheese to ripening changes and concentration of chemical products. In this treatment, the grade of the cheese and the characteristic Cheddar flavor intensity were related to each other and to pH, ammonia, hydrogen sulfide, individual free fatty acids, individual acidic and neutral carbonyl compounds, individual free amino acids, and bacterial counts. A total of 61 correlations was made for each of five curing periods (0.5, 3, 6, 9, and 12 mo.). Results are in Table 6.

With respect to the grade of the cheese, the pH relationship appeared to be the only one with any degree of consistency (significant for three of the five periods). Many of the other relationships reached a statistically significant level at one time or another during curing. However, these relationships were temporary only (C.F. at 0.5 mo., ammonia at 6 mo., acetic acid at 0.5 mo., phenylalanine at 3 mo.).

The coefficient of correlation values indicate a relatively consistent relationship between characteristic Cheddar Cheese flavor values and three ripening changes—pH, ammonia, and hydrogen sulfide. Of these, the flavor–hydrogen sulfide correlation was positive at all periods, and at 6, 9, and 12 mo. the relationship was significant at the 1% level. The pH and ammonia relationships were significant for three out of five curing periods. The Cheddar flavor relationship with free fatty acids and free amino acids were inconsistent for the various curing periods. Similarly, no consistently significant relationships appeared to exist between the grade and the characteristic Cheddar flavor to the other amino acids (not included in Table 6), nor to the neutral and acidic carbonyl compounds and bacterial counts.

Free fatty acids have been associated previously with development of desirable flavor in Cheddar Cheese (14, 19). The lack of a statistically significant relationship between the quality of the cheeses and the free fatty acids content in this study may be explained by the observations made by Kristoffersen *et al.* (8), that (a) relatively high concentrations of free fatty acids are associated with cheese of less desirable characteristics, and that (b) low-quality cheeses possessing high concentrations of free fatty acids were generally low in hydrogen sulfide. These observations suggest a possible relationship between the rate of appearance and actual concentrations of both free fatty acids and hydrogen sulfide to characteristic Cheddar flavor development. The observations in the present study which suggest this relationship include (a) the high correlation of hydrogen sulfide concentrations to characteristic flavor, (b) the approxi-

TABLE 6

Statistical correlation of the grades and the characteristic Cheddar flavor values (C.F.) of commercial Cheddar Cheese to selected ripening changes and products

Age (mo.)	Relationship	r (coefficient of correlation)[a]										
		C.F.	pH	NH_3	H_2S	Acetic Acid	Propionic Acid	Butyric Acid	Other fatty acids	Glutamic Acid	Alanine	Phenylalanine
0.5		−.62	.23	.38	−.18	.79	.52	−.04	.30	.22	.10	−.25
3		−.34	.61	.28	.11	−.16	.33	.40	.36	.26	.10	−.73
6	Grade	.27	.48	.61	.23	.05	.37	.35	.50	.37	−.03	−.20
9		.05	.57	.49	.33	.34	.40	.52	.64	.04	.45	.47
12		−.06	.62	.34	.19	.05	.39	.60	.49	−.25	−.19	−.12
0.5		1.00	−.32	−.23	.38	−.43	.48	.20	.22	.06	.14	.40
3	Characteristic	1.00	.33	.51	.59	.08	.15	.12	.32	−.12	.21	.29
6	Cheddar	1.00	.66	.73	.66	.16	.56	.51	.60	.36	.13	−.07
9	flavor (C.F.)	1.00	.65	.65	.73	−.07	.22	.27	.34	−.03	.08	−.25
12		1.00	.57	.48	.79	−.10	.25	.38	.35	.03	.12	.20

[a] r = 0.51—significant at the 5% level.
r = 0.64—significant at the 1% level.

mately equal concentrations of free fatty acids in some cheeses of both high and relatively low characteristic flavor values, and (c) the almost complete absence at all periods of characteristic flavor in Lot 14 which had an apparent normal protein breakdown but an abnormal formation of free fatty acids.

To demonstrate this relationship, ratios of concentrations of total free fatty acids to hydrogen sulfide are presented in Table 7. The ratios are for three lots

TABLE 7

Relationship of the ratio of total free fatty acids to hydrogen sulfide and characteristic Cheddar flavor in selected commercial Cheddar Cheeses

Age in months	Lot 1		Lot 11		Lot 7	
	f.f.a.[a] H_2S	C.F.[b]	f.f.a.[a] H_2S	C.F.[b]	f.f.a.[a] H_2S	C.F.[b]
0.5	6.2	2	21.7	0	7.0	1
1.5	7.0	..[c]	15.8	..[c]	3.0	..[c]
3	8.7	6	21.3	1	7.7	1
6	7.1	4	20.2	3	3.8	3
9	9.8	5	19.8	3	4.8	4
12	12.7	5	24.8	2	5.1	4

[a] Micromoles of total free fatty acids per gram/micromoles of hydrogen sulfide per 100 g. of cheese.
[b] Characteristic Cheddar flavor.
[c] Not evaluated.

representative of cheeses with (a) high total free fatty acids—high hydrogen sulfide concentrations (Lot 1); (b) high total free fatty acids—low hydrogen sulfide concentration (Lot 11); and (c) low total free fatty acids—high hydrogen sulfide concentrations (Lot 7). Ratio values ranged from 6.2 to 12.7 in Lot 1, from 15.8 to 28.4 in Lot 11, and from 3.0 to 7.0 in Lot 7. Roughly, the ratios observed in Lot 1 were one-half of those in Lot 11 and twice those of Lot 7. Generally, Lot 1 received the highest flavor scores, followed by Lot 7 and then Lot 11. The flavors of these three lots, although all typical Cheddar, were still distinctly different. The flavor of Lot 1 was usually considered blended, whereas, that of Lot 11 was suggestive of free fatty acids and that of Lot 7 of hydrogen sulfide.

On the basis of this preliminary comparison, the 14 lots of cheese were divided into three groups and categorized as to flavor-type: (a) balanced, (b) sulfide-like, or (c) fatty acid–like. The data in Table 8 indicate the number of cheeses in each group and the total free fatty acids—hydrogen sulfide ratios and characteristic Cheddar flavor values at 12 mo. Raw milk cheese predominated in the balanced flavor group and pasteurized milk cheese in the fatty acid–like group.

The ratios of total free fatty acids to hydrogen sulfide ranged from 17.6 to 11.7; 9.3 to 5.1; and 36.8 to 22.0 in the balanced, sulfide-like, and fatty acid–like groups, respectively. The average ratio values were 14.2, 7.2, and 27.9. Thus, as indicated in Table 7, the ratios of the balanced flavored cheeses were approximately twice as high as those of the sulfide-like flavored cheeses, but only one-half as great as those for cheese with fatty acid–like flavor.

TABLE 8

Type of Cheddar Cheese flavor as related to the ratios of free fatty acids to hydrogen sulfide at 12 mo.

Type of flavor	Cheese[a] No.	Milk treatment	Fatty acids[b] H_2S	Quality C.F.	Quality Grade
	2	Raw	17.6	4	B
	4	Raw	16.1	6	B
Balanced	8	Pasteurized	13.1	5	A
	1	Raw	12.7	5	B
	3	Raw	11.7	5	C
	Average		14.2	5	..
	5	Raw	9.3	6	A
Sulfide-like	14	Pasteurized	8.7	1	C
	6	Heated	5.7	5	A
	7	Pasteurized	5.1	4	B+
	Average		7.2	4	..
	9	Pasteurized	36.8	5	B+
	10	Pasteurized	30.0	4	B
Fatty acid-like	12	Pasteurized	26.1	3	B−
	11	Pasteurized	24.8	2	C
	13	Pasteurized	22.0	3	C
	Average		27.9	3.4	..

[a] Arranged in decreasing order of ratio values.
[b] Micromoles of total free fatty acids per gram/micromole of hydrogen sulfide per 100 g. of cheese.

With the exception of Cheese 14, there appeared to be little difference in the acceptance of balanced and sulfide-like cheese with respect to characteristic Cheddar flavor. Both groups were rated high at most curing periods.

The characteristic flavor values of the fatty acid-like cheese were generally lower throughout curing than those of the other two groups. At 12 mo. the values ranged from 5 to 2, with an average value of 3.4. Cheese 9, with a superior flavor, was an exception to the general trend among cheeses in this group.

Determination of the total free fatty acids to hydrogen sulfide ratio during the early history of a Cheddar Cheese may offer some possibility for establishing its ripening potential, i.e., its future flavor intensity and type. Exceptions can be expected, however. Of the 14 lots in this study, two did not develop flavor as might be predicted from the ratios obtained at 0.5 mo. of curing. At this time, Cheese 7, on the basis of the fatty acids—hydrogen sulfide ratio, appeared to be a balanced flavored cheese, but it changed to sulfide-like after about 3 mo. of curing. Cheese 8 initially was fatty acid–like, based on both flavor and analytical evaluations; however, from the 3-mo. period it was one of the better balanced flavored cheeses.

DISCUSSION

The present study differed from the previous one in this series, in that the commercial cheese was obtained fresh and was then cured under controlled conditions. This restricted the experimental variables to the milk supply and manufacturing methods. The present study also differed from those reported by other workers, in that commercial and not laboratory cheese was used. The results presented, coupled with the previously published findings on carbonyl

compounds (7), show clearly those ripening products which are produced in appreciable quantities through fat and protein degradation during curing of Cheddar Cheese.

Previously, attempts have been made to relate Cheddar Cheese flavor to breakdown products of either fat (6, 11, 12, 14, 17, 19) or protein (1, 3, 5, 6, 9, 10, 17, 18, and 20). The evidence presented in the present paper suggests strongly that optimum flavor in Cheddar Cheese results only through simultaneous action of the agents responsible for fat and protein degradation. In addition, the speed with which these agents act probably is a critical factor.

The balance of compounds theory with respect to characteristic cheese flavor has been developed in recent years through research on Romano, Provolone, and Swiss cheese (2, 4, 13). The development of characteristic flavor in these varieties was related to simultaneous appearance of certain free fatty acids and free amino acids. The importance of fat degradation in Cheddar Cheese has been indicated (6, 12, 14, 15, 17, 19). Therefore, it would appear only logical to include products of fat degradation as one part of a flavor index for Cheddar Cheese. On the basis of the results in the present study, the total free fatty acid content was selected for this purpose, as the flavor of Cheddar Cheese did not suggest the presence of exceptionally high concentrations of one specific free fatty acid, as did the flavors of Romano, Provolone, and Swiss cheese.

With respect to selection of the second part of the flavor index, i.e., that resulting from protein degradation, it appears logical to select hydrogen sulfide because of (a) its distinct flavor, (b) its proven importance to flavor in other food products (16), (c) the relatively restricted sources of origin (cystine and methionine), (d) the relative ease of determination, and (e) the high degree of statistical correlation between the concentrations of this compound and characteristic Cheddar flavor.

Although the present study indicates the importance of simultaneous breakdown of fat and protein to the development of characteristic Cheddar flavor, and that free fatty acids and hydrogen sulfide may contribute directly to this flavor, it does not indicate which factors are responsible for these changes. The next area deserving attention concerning Cheddar Cheese flavor appears to be that of the factors which control the development of flavor.

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METHANOL-SOLUBLE COMPLEXES OF LACTOSE AND OF OTHER CARBOHYDRATES

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SUMMARY

Lactose, sucrose, maltose, galactose, fructose, and many other carbohydrates and derivatives are found highly soluble in cold absolute methanol containing sufficient calcium chloride. From a highly concentrated viscous solution prepared from lactose, calcium chloride, and methanol in a molar ratio such as 1:3:24, a complex of β-lactose, calcium chloride, and methanol in the molar ratio of 1:1:4 slowly crystallizes. After about 6 *M* of water are added to such a concentrated solution per mole of calcium chloride, a complex of α-lactose, calcium chloride, and water in the molar ratio 1:1:7 soon crystallizes. The equilibrium ratio of α- to β-lactose in the concentrated solutions is about 5:3. The dilution of such a concentrated solution with methanol causes lactose to crystallize in the same anomeric ratio as exists in the solution. Within the investigated range, the logarithm of the solubility of this crystallized lactose in methanol is a linear function of the calcium chloride concentration.

Complexes of calcium salts, sugars, and water were first described by Ritsert (23, 24), who prepared a crystalline product consisting of 1 *M* of lactose, 1 *M* of calcium chloride, and between 6 and 8 *M* of water, and who later investigated similar addition products of other sugars. Many other investigators later prepared many other complexes of sugars and hydrated salts of alkali or alkaline earth metals (1, 6, 7, 8, 12, 13, 20, 21, 25, 26). The properties of the complex containing lactose, calcium chloride, and water of hydration were investigated by Herrington (9).

As alcohols, like water, are able to solvate calcium chloride (3, 4, 16, 17, 18, 19), we thought it might be of interest to investigate the analogous sugar-calcium chloride-alcohol addition products; such products had never been prepared. The compound that Dale (6) prepared by evaporating slowly a solution of mannose and calcium chloride tetrahydrate in hot ethanol contained water rather than ethanol; it was β-mannose·$CaCl_2$·$2H_2O$. We have found that many sugars, even anhydrous, are highly soluble in solutions of calcium chloride and anhydrous methanol. Of the various sugars used, lactose was the most thoroughly investigated.

EXPERIMENTAL METHODS

Gross composition. Calcium chloride was determined by ashing, followed by analyses for calcium by permanganate titration of the precipitated oxalate (14), and chlorine by Volhard's procedure (15). Volatiles, taken to be either methanol or water, depending on how the particular solution or crystals had been prepared, were determined by drying at 60° C. for 24 hr., and then at 105° C. to constant weight. Lactose was determined either by difference or by Munson-Walker test.

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Vapor pressure and solvation levels. The vapor pressures of solvation levels of crystalline complexes were measured by the static method of Bagster (2). A small flask containing the test substance was connected with a manometer and provided with stopcocks. The entire apparatus was kept in a cabinet at 35.5° C. The system was successively evacuated for various time intervals and allowed to come to equilibrium again for about 1 hr. After each such equilibration, the pressure was read, dry air was admitted to the flask, and a small sample of test substance was removed to allow determination of its residual methanol content.

Polarimetry. Rotations were determined in water at carbohydrate concentrations between 1.5 and 3%. Both final (equilibrium) rotations and initial (i.e., extrapolated to zero time) rotations were measured. The small concentrations of calcium chloride and methanol present in some of the aqueous solutions measured were found to have a small, generally negligible effect on the optional rotations. Temperatures higher than 60° C. were avoided whenever a sample containing calcium chloride was dried.

Chromatography. One gram of the sample dissolved in 10 ml. of water was deionized through a mixed bed column (27). The aqueous eluate was evaporated in a warm air current at 40° C. The residue was dissolved in an aqueous solution containing 10% of isopropyl alcohol. Paper chromatographic development was carried out, using the descending technique with water-saturated phenol as the developing agent. Following a 24-hr. resolution the spots were made visible (5).

EXPERIMENTAL PART AND DISCUSSION

Highly concentrated solutions of lactose in methanolic calcium chloride. Such solutions were prepared from various forms of lactose and with many variations in concentrations. Typically, 100 g. of anhydrous calcium chloride were dissolved in 300 g. of absolute methanol. To the cooled solution, 100 g. of anhydrous lactose were added; this mixture was stirred at 40° C. till nearly all dissolved.

Generally, the solid form of lactose used was the stable anhydrous nonhygroscopic crystalline form. This was prepared according to the directions of Herrington (10), which call for merely letting α-lactose hydrate stand in methanol.

After the methanolic solution had remained at room temperature for several days, the change of rotation after dilution with water was determined. The ratio of initial to final rotation of the aqueous solution always approached the value 1.3, regardless of whether the lactose originally dissolved in methanol and calcium chloride had been the α- or β-isomer.

The compositions of three such solutions are shown in Table 1 (No. 1, 2, and 3). Two of these were prepared from stable α-lactose anhydride and one from β-lactose. The initial and final specific rotations, as well as the ratio of these rotations, are about equal.

To explain the low final rotation of these solutions, one might speculate that some unknown compound having a large negative specific rotation might be

TABLE 1

Polarimetry and composition of products

No.	Type	Mode of preparation	Composition, Lactose	Composition, Wt. % $CaCl_2$	Specific rotation of lactose, Initial	Specific rotation of lactose, Final	Specific rotation of lactose, Initial/Final
1	Solution	Lactose[a] in (MeOH+$CaCl_2$)	24.6	18.1	54.9	42.3	1.30
2	Solution	Lactose[b] in (MeOH+$CaCl_2$)	19.5	19.8	57.3	43.8	1.30
3	Solution	Lactose[a] in (MeOH+$CaCl_2$)	19.5	24.3	57.3	44.4	1.29
4	Complex	Crystd. from No. 1	47.7	18.0	41.6	50.6	0.83
5	Complex	Crystd. from No. 2	44.6	22.5	45.6	51.7	0.88
6[c]	Solution	(Lactose[a] + $CaCl_2$ + MeOH) in H_2O	47.0	18.0	90.7	56.3	1.61
7[d]	Solution	(Lactose[b] + $CaCl_2$ + MeOH) in H_2O	45.0	22.0	41.2	56.3	0.73
8	Lactose	Crystd. from (No. 4 in MeOH)	99.3	0.7	45.6	54.8	0.83
9	Lactose	Crystd. from (No. 1 + MeOH)	99.8	0.2	69.3	54.2	1.28
10	Lactose	Crystd. from (No. 2+ MeOH)	99.9	0.1	69.7	54.7	1.28
11	Lactose	Pptd. from (No. 1 + EtOH)	82.1	15.0	68.7	54.7	1.27
12	Lactose	Pptd. from (No. 2 + EtOH)	79.5	17.9	69.7	54.7	1.27
13	Complex	Crystd. from (No. 1 + H_2O)	57.7	17.9	73.3	50.3	1.46

[a] Stable anhydrous crystalline α-lactose.
[b] Commercial impure β-lactose.
[c] Control for polarimetry of No. 4.
[d] Control for polarimetry of No. 5.
[e] The difference from 100% would represent water for No. 13, ethanol for No. 11 and 12, and methanol for all others.

present. By chromatography, however, the total concentration of compounds other than lactose was conservatively estimated at less than 3% of the lactose concentration. Some of these compounds had R_f values close to those of the compounds (22) which are formed during the enzymatic disproportionation of lactose. These latter have specific rotations lower than that of lactose, but not low enough to explain the final rotations of the lactose solutions No. 1, 2, and 3.

β-Lactose-calcium chloride-tetramethanol crystalline complex. From the highly concentrated methanolic solution, heavy crystallization occurred after months, or when it was seeded and shaken, after several days. The very small crystals were separated by prolonged centrifugal filtration in a closed system, since they could not be washed without change in composition. The crystals were dried first at 60° C. to avoid the melting and decomposition which would otherwise have occurred when the rest of the methanol was finally evaporated at 105° C.

Even though they were thus carefully dried, their initial optical rotation changed when they were heated at length above 100° C., and gradually approached their final rotation. Montgomery and Hudson (20) found, similarly, that the rotation of arabinose-calcium chloride complexes changed when heated.

Chromatography of the crystals showed that the only carbohydrate present in significant proportion was lactose. Polarimetry showed it was mainly the β-isomer (Table 1, No. 4-7). The molar ratios of calcium chloride to lactose in five products were 1.06, 1.16, 1.03, and 0.96 when crystallized at room temperature, and 1.38 when crystallized in the cold. The methanol content varied between 21 and 31% (Calcd. for $C_{12}H_{22}O_{11} \cdot CaCl_2 \cdot 4CH_3OH$, 22%).

It is reasonably sure that the crystalline complex contains lactose and calcium chloride in the molar ratio of unity. The high ratio 1.38 found for one product must have been caused by the crystallization, too, of some of the calcium chloride-methanol complex ($CaCl_2 \cdot 4CH_3OH$), which was sometimes seen to crystallize first when such solutions were chilled.

The solvation level of the centrifugally filtered crystals and also lower levels were determined by vapor pressure measurements. The successive vapor pressures and analyses indicate that lactose$\cdot CaCl_2 \cdot CH_3OH$ gave up its first molecule of methanol between 130 and 136 mm., and its second at 19-25 mm.; it then retained two molecules of methanol.

That the crystalline phase consists of a coordinated complex rather than an intimate mixture of the components is made probable by its solubility in methanol. While one gram of complex was completely soluble in 50 ml. of methanol, an intimate mixture of lactose and calcium chloride, in the same ratio as present in the complex, was not.

Methanol decomposed the complex; the complex first dissolved, and soon lactose itself crystallized from the clear solution. Obviously, at this concentration more than 1 *M*-equivalent of calcium chloride would be needed to hold each mole-equivalent of lactose in solution. The methanol and lactose must compete in solution for coordination with calcium chloride, while only those lactose molecules which are not coordinated can be directly in dynamic equilibrium with crystals of pure lactose.

The lactose which crystallized from the solution of the complex which had been redissolved in methanol had an initial specific rotation of 45.6° and a final specific rotation of 54.8° (see Table 1, No. 8). Although the initial specific rotation of this crystallized lactose is quite similar to that of lactose coordinated with calcium chloride in a complex (Table 1, No. 4 and 5), it is quite different from that of lactose caused to crystallize by diluting with methanol a concentrated solution of lactose in methanolic calcium chloride (No. 9 and 10).

In the crystalline complex lactose$\cdot CaCl_2 \cdot 4CH_3OH$ the lactose could be bound to the calcium ion directly through coordinate covalent bonds, or indirectly through hydrogen bonds with the methanol molecules that are coordinated with calcium. In the hydrated crystalline complex (9) lactose$\cdot CaCl_2 \cdot 7H_2O$, on the other hand, the calcium is probably coordinated with only six of the molecules of water, since its maximum coordination number is six.

Precipitation of lactose by methanol. If more methanol is added to a concentrated solution of lactose and calcium chloride in methanol, lactose can be caused to crystallize. This crystallization can be hastened by seeding and shaking. Several solutions were diluted with various proportions of methanol. After the solutions were shaken for some time at 28° C., lactose began to precipitate. After about 2 wk., the refractive index of the mother liquor remained constant for several days; the crystallized lactose was then separated and the compositions of each solution and of several of the corresponding precipitates were determined.

When the logarithm of the lactose concentration in solution is plotted

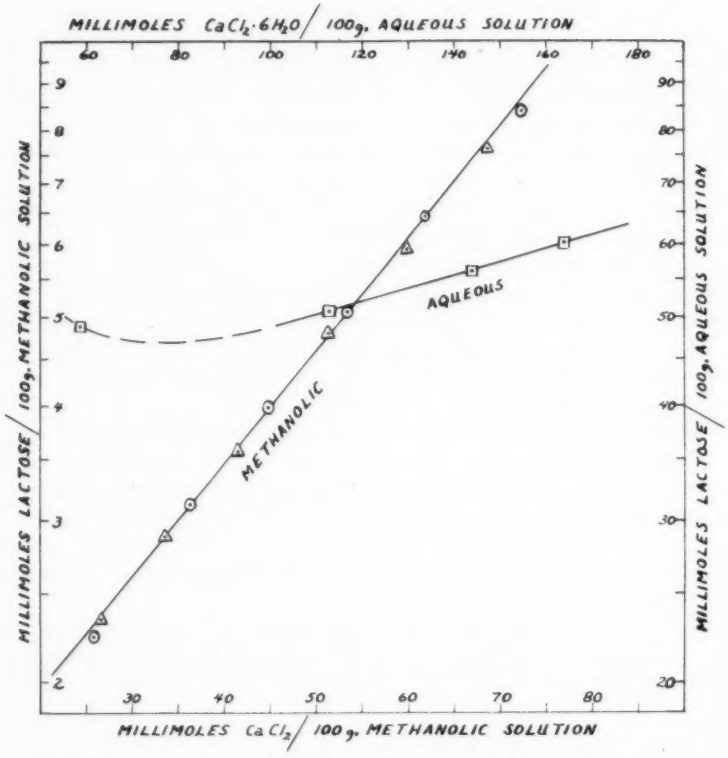

FIG. 1. Solubility of lactose in methanolic calcium chloride and in aqueous calcium chloride. Saturated solutions represented by circles and triangles were obtained by dilution of solutions having the respective compositions (in weight %): lactose 19.35, $CaCl_2$ 21.45, CH_3OH 59.20; lactose 19.41, $CaCl_2$ 21.12, CH_3OH 59.19. The solutions represented by squares were obtained by Herrington (9).

is obtained. Its equation is

$$\log [\text{lactose}] = 0.04 + 0.01211 [CaCl_2]$$

(Figure 1) against the concentration of calcium chloride, a nearly straight line where the concentrations of lactose and calcium chloride are in millimoles per 100 g. of solution.

Herrington (9) determined the solubilities of lactose and of the complex lactose·$CaCl_2 \cdot 7H_2O$ in aqueous calcium chloride solutions. If one plots (see Figure 1) the results of his four tests in which lactose was the solid phase, the logarithm of the lactose concentration turns out to be another straight line function of the calcium chloride concentration for the highest three points; the lowest point is off the line.

The lactose samples crystallized by diluting the concentated solutions with methanol (Table 1, No. 9 and 10) had final specific rotations of about 54.5°,

as expected for anhydrous lactose, but the initial specific rotations were about 69.3°. The ratio (about 1.28) of initial to final rotation was close to the ratio (1.3) for the solutions (No. 1 and 2) from which these samples crystallized.

When the concentrated solutions were diluted with ethanol instead of methanol, precipitates (Table 1, No. 11 and 12) were formed at once. They were removed by centrifugation. Their composition indicated that some lactose·$CaCl_2$·$4CH_3OH$ complex was coprecipitated with lactose. The initial and final specific rotations of their lactose content were the same as those of the pure lactose (No. 9, 10) which crystallized when methanol was the diluent.

Hockett and Hudson (11), by letting α-lactose monohydrate stand in contact with methanol containing between 1 and 5% of hydrogen chloride, obtained a crystalline lactose species having an initial rotation of 67.9° and a final rotation of 54.2°. They considered the crystals to be a molecular compound consisting of α-lactose and β-lactose in a molar ratio of 5:3. Their ratio of initial to final rotation, 1.23, was somewhat lower than that of the lactose which crystallized from the diluted methanolic calcium chloride solutions. This difference might be explained by assuming that while either calcium chloride or hydrogen chloride may act as a mutarotation catalyst, a small change in the mutarotation equilibrium of lactose in methanol is caused by the presence of high concentrations of calcium chloride.

Lactose-calcium chloride heptahydrate complex. When about 6 *M* of water were added to the methanolic lactose solution (prepared either from the α or β form) for each mole of dissolved calcium chloride, the maximal amount of a complex consisting of lactose·$CaCl_2$·$7H_2O$ gradually crystallized over a period of a few days. Adding either more water or less lowered the yield of crystals. The start of crystallization could be hastened by seeding with crystals from a previous batch. Although these crystals, as deposited from methanol, did not look so large and well formed as those deposited from water according to Herrington's procedure (9), both appeared to have the same crystalline form. The molar ratio of lactose to calcium chloride was 0.97, and the compounds contained slightly more water than the compounds investigated by Herrington. (Water was found to be the major component of the volatile phase.) The measurements of initial and final specific rotation showed that the lactose part was mostly in the α form.

Solubilities of several polyhydroxy compounds. In general, monoses, bioses, trioses, and some of their simple derivatives were found soluble in methanolic calcium chloride; only the higher polysaccharides were insoluble. These qualitative solubilities were determined at 28° C. by mixing the carbohydrate with 1.5 *M* of calcium chloride and 12 *M* of methanol per hexose unit.

The following were soluble: D-sorbitol, lactositol, D-glucono-δ-lactone, α-methyl-D-glucoside, maltose (hydrate), L-sorbose, melezitose, D-galactose, sucrose, and lactobiono-δ-lactone. These dissolved but later crystallized: D-fructose, raffinose, dry amorphous D-glucose, and several forms of lactose (α-hydrate, desiccated α-hydrate, stable anhydrous α, β, and a noncrystalline spray-dried solution). Dextrin and dextran were insoluble. Seemingly without dissolving,

crystalline D-glucose changed into a solvated calcium chloride (1:1) complex. Calcium bromide, too, though not zinc chloride, brought lactose into solution in methanol.

The fructose solution was of special interest in that soon after it began to crystallize it became a translucent homogeneous rigid gel which filled the entire volume of the solution. Cutting or pressing the gel caused opaque white surfaces to appear. These crystals were a difructose-calcium chloride-tetramethanol complex (calcd. for $2C_6H_{12}O_6 \cdot CaCl_2 \cdot 4CH_3OH$: Ca 8.50, CH_3OH 21.4; found: Ca 8.57, CH_3OH 21.5). Fructose has been known (28) to give a somewhat similar gel in water.

Methanolic calcium chloride might serve as a solvent for sugar reactions, and for fractionating sugar mixtures by extraction or crystallization.

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SPECIFICATIONS FOR THE SPREADABILITY OF BUTTER [1]

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SUMMARY

Specifications for the spreadability of butter have been established in objective terms. A wide range of resistance to spreading has been obtained by tempering the butter samples at different temperatures. Spreadability measurements made with a modification of the Huebner-Thomsen apparatus are recorded. These data have been compared with the subjective assessments of a panel of 15 persons. The objective spreading resistances corresponding to the subjective desirable spreadability ranged between 260 and 530 g. The range for acceptable spreadability was 160 to 890 g. In the single brand of margarine tested, the desirable spreadability ranged between 260 and 540 g. and the acceptable spreadability ranged between 160 and 1,100 g.

In recent years, with the greater emphasis on rheology in the food industry, the spreadability of butter has become increasingly important. Although instruments to measure the firmness of butter had been used as early as 1891, spreading instruments are relatively new. Heretofore, no specifications were available for the spreadability of butter. Such quantitative specifications should be helpful in appraising various treatments said to improve the spreadability of butter and in setting up optimum limits for this property of butter.

The aim of this study was to establish specifications for the spreadability of butter in objective terms.

The methods used to measure objectively the rheological properties of butter are dependent on penetration, compression, sectility, sagging, extrusion, or spreading. The first four methods have been devised to measure firmness or hardness of butter, whereas extrusion and spreading methods are used to measure spreadability.

With the extrusion method the product is forced through one or several small openings and the required pressure is recorded. This principle was first used by Gardner and Van Heuckeroth (3) and later by Griffiths (4), Arveson (1), Sargent (8), Mohr and Häsing (6), and Prentice (7). This last worker has incorporated the principle into a compact instrument, in which the butter sample is forced at constant rate through a small orifice. The thrust on the piston is graphically recorded. The thrust at the moment of emptying is taken as the resistance to extrusion.

In spreading we are concerned with the friction between the particles of fat when one layer slides rapidly over another. The first spreading instrument was developed in 1937 by De Lacy (2). Its main features were a trolley to which a blade was attached and which was pulled by weights. Mechanical friction and unevenness of speed were the main drawbacks of this instrument. Mohr and Häsing (6) modified De Lacy's instrument. The beveled blade attached

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[1] Contribution No. 35.

to a trolley was pulled at a constant speed of 1 cm/sec. The resistance to spreading was measured on a dynamometer placed between the trolley and the pulling string. These basic principles were incorporated into the instrument developed in 1957 by Huebner and Thomsen (5), and later modified. A constant-speed motor is used to pull the knife at the speed of 1 cm/sec. Friction is minimized by suspending the spreading knife at the end of a 61-cm. arm. The beveled blade (1.6 mm.) is thinner than that used by Mohr and Häsing (2 mm.). The butter area exposed to the spreading blade is half the size (3.2 by 7.6 cm.) of that used by Mohr and Häsing. The shims used to raise the sample between measurements are 1.6 mm. thick instead of the 2-mm. shims used by Mohr and Häsing. A calibrated torque indicator placed between the blade and the motor registers the resistance to spreading.

METHODS

The modified Huebner-Thomsen apparatus was used in this study because it is more sensitive than the extrusion methods (5, 7) and is more representative of the actual spreading of butter.

A series of 57 butter samples and 38 margarine samples was examined with the spreadability apparatus and at the same time organoleptically by a panel of 15 persons. The butters used were 1-lb. prints which were available from churnings made at the Institute Dairy in connection with another experiment. The margarine samples, all of a single brand, were obtained from a local store. The spreadability instrument was kept in a room where the temperature was controlled at $\pm 0.5°$ C. The prints of butter and margarine to be examined were tempered in this room at least 48 hr. before testing. Various degrees of spreadability and hardness were obtained by changing the temperature of the testing room over the range of 4 to 22.5° C.

On a testing day the spreading resistance of the butter or the margarine sample was measured with the spreadability instrument. Eight readings were made on each sample. The average standard deviation for the measurements below 1,000 g. was 3.2. Above 1,000 g. the variations were larger and the standard deviation was in the order of 15 g. Huebner and Thomsen (5) reported a standard deviation of 4.3 on the instrument before its modification.

The 15 judges of the panel were members of the Institute staff who had had previous experience on organoleptic panels but not on spreadability. They were asked to assess the handling characteristics of each print by feeling with fingers or with a knife, and the spreadability by spreading some of the sample on fresh bread with a table knife. Handling and spreadability each were to be described by one of the following five terms: much too hard, hard but acceptable, desirable, soft but acceptable, much too soft. Numerical rating was not used to distinguish between too soft and too hard.

To convert the descriptive terms into numerical values the following factors were assigned to each: 1 for much too soft, 2 for soft but acceptable, 3 for desirable, 4 for hard but acceptable, and 5 for much too hard. The numerical limits for each descriptive term are as follows: 1.0-1.5 for much too soft, 1.5-2.5

for soft but acceptable, 2.5–3.5 for desirable, 3.5–4.5 for hard but acceptable, and 4.5–5.0 for much too hard.

In establishing the specifications for spreadability the handling characteristics of the print were taken into consideration. For the appraisal of the handling properties of the print the panel members were asked to consider if the sample was firm enough to keep its form when handled at the store or at home, and if it was soft enough to be cut with a knife, in pats or otherwise.

RESULTS AND DISCUSSION

The resistance to spreading for the 57 butter assessments ranged from 80 to 1,380 g. Figure 1 shows that the spreadability of butter was described as

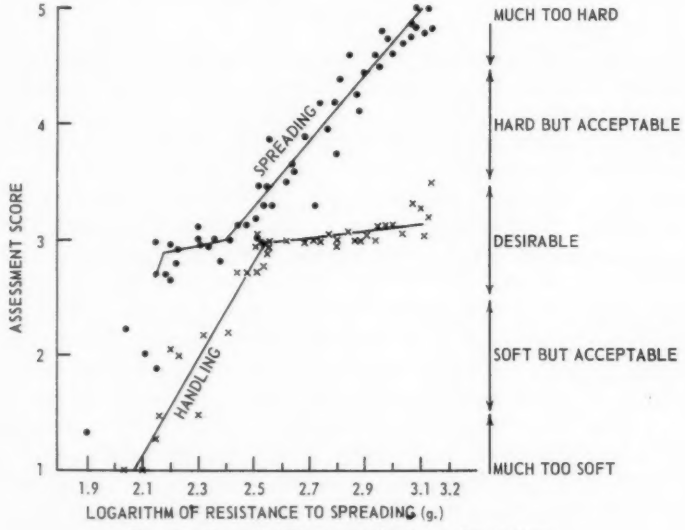

FIG. 1. Spreadability specifications for butter.

desirable from 130 to 530 g. (logarithms 2.1 and 2.7) of resistance to spreading, but that the handling was described as desirable from 260 to 1,380 g. (logarithms 2.4 and 3.1) of resistance. Desirable spreadability was limited at one end by handling and at the other end by spreading. Accordingly, the specifications for desirable spreadability were 260 to 530 g. of resistance. Similarly, the specifications for acceptable spreadability were 160 to 890 g. of resistance.

A straight-line relationship, in agreement with the results of Prentice (7), was observed between the spreadability assessment and the logarithm of resistance to spreading. The correlation coefficients were 0.94 for spreading and 0.93 for handling. Huebner and Thomsen (5) reported that a change of one rating unit was equivalent to 314 g. of resistance to spreading. Our average resistance gradient was about the same (320 g.) but was not uniform. It increased with

the resistance to spreading. It was 100 g. for soft but acceptable, 280 g. for desirable, and 560 g. for hard but acceptable.

In Table 1, the percentages of judgments for both spreading and handling of butter were calculated for each range of specifications. Below 160 g. of

TABLE 1

Subjective assessments of butter for desirable and acceptable spreadability ranges

Range of spreadability resistance		Much too hard	Hard but acceptable	Desirable	Soft but acceptable	Much too soft
(g.)		*(% judgments)*				
Acceptable range:						
80-160	Spreadability	0	0	29.4	35.6	35.0
(much too soft)	Handling	0	0	1.6	14.5	83.9
160-890	Spreadability	12.6	47.4	40.0	0	0
(acceptable)	Handling	0	3.9	72.2	18.0	5.9
890-1380	Spreadability	78.5	21.5	0	0	0
(much too hard)	Handling	0.6	19.3	80.1	0	0
Desirable range:						
80-260	Spreadability	0	0.7	62.8	20.9	15.6
(soft)	Handling	0	0	10.3	44.1	45.6
260-530	Spreadability	0	30.1	69.9	0	0
(desirable)	Handling	0	0	88.6	9.9	1.5
530-1380	Spreadability	49.3	44.1	6.6	0	0
(hard)	Handling	0.3	14.2	85.0	0.5	0

resistance butter was too soft to be acceptable and this was influenced mainly by the handling characteristics. From 160 to 890 g. the butters were in the acceptable range and above 890 g. they were much too hard, mainly on account of being difficult to spread, although the handling characteristics were desirable. Below 260 g. of resistance the butters were not firm enough to be in the desirable

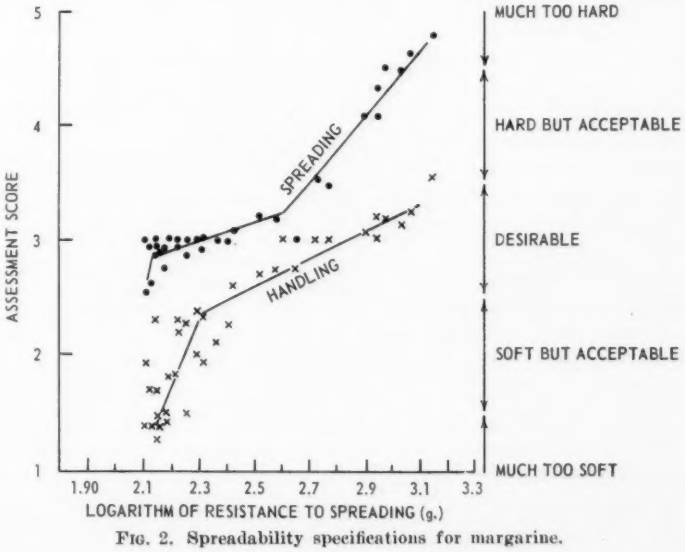

FIG. 2. Spreadability specifications for margarine.

or optimum range of resistance, 260 to 530 g. Above 530 g. the butters were desirable for handling but too hard for spreading.

The spreadability resistances for the 38 margarine assessments ranged between 130 and 1,410 g. Figure 2 illustrates that the desirable spreading ranged from 130 to 540 g. (logarithms 2.1 and 2.7), whereas the desirable handling ranged from 260 to 1,400 g. (logarithms 2.4 and 3.1). Consequently, the specifications for desirable spreadability were 260 to 540 g. of resistance. The range is similar to that obtained with butter. The limits for acceptable spreadability were 160 and 1,100 g. The correlation coefficients between the logarithm of the resistance to spreadability and the assessment score were 0.96 for spreading and 0.86 for handling.

Table 2 gives the percentages of judgment for each range of specifications. Below 160 g. the margarine was quite desirable for spreading but not acceptable

TABLE 2

Subjective assessments of margarine for desirable and acceptable spreadability ranges

Range of spreadability resistance		Much too hard	Hard but acceptable	Desirable	Soft but acceptable	Much too soft
(g.)		*(% judgments)*				
Acceptable range:						
130-160	Spreadability	0	0	85.0	15.0	0
(much too soft)	Handling	0	0	5.4	47.4	47.2
160-1100	Spreadability	8.3	16.3	73.8	1.6	0
(acceptable)	Handling	0.3	1.8	50.8	41.0	6.1
1100-1410	Spreadability	74.5	25.5	0	0	0
(much too hard)	Handling	0	39.6	60.4	0	0
Desirable range:						
130-260	Spreadability	0	0	91.5	8.5	0
(soft)	Handling	0	0	12.6	59.4	28.0
260-540	Spreadability	0	21.2	78.8	0	0
(desirable)	Handling	0	0	79.8	20.2	0
540-1410	Spreadability	44.6	41.5	13.9	0	0
(hard)	Handling	0.8	15.5	83.7	0	0

for handling. Between 160 g. and 1,100 g. the margarine was classed acceptable and above 1,100 g. it was found too difficult to spread.

The analysis of covariance indicated that the curves for butter and margarine were not common. The slopes for the spreading curves were identical. Apparently, the panel members were slightly more critical of butter than of margarine.

Although the previous histories of the butter and margarine samples were not known, the spreading resistances were plotted against temperatures. Figure 3 indicates that desirable spreadability occurred at 15-18° C. for butter and at 11-14° C. for margarine. Consequently, both butter and margarine samples were too hard to be desirable at the temperatures of the home refrigerator (4-7° C.). The spreadability of margarine, however, was acceptable at 7° C. Above 17.5° C. the margarine samples were too soft to be acceptable, whereas the butter samples reached that condition only above 20.5° C.

The objective in improving the spreadability of butter should be to produce firm butter with acceptable spreadability (resistance of 160-890 g.) or preferably

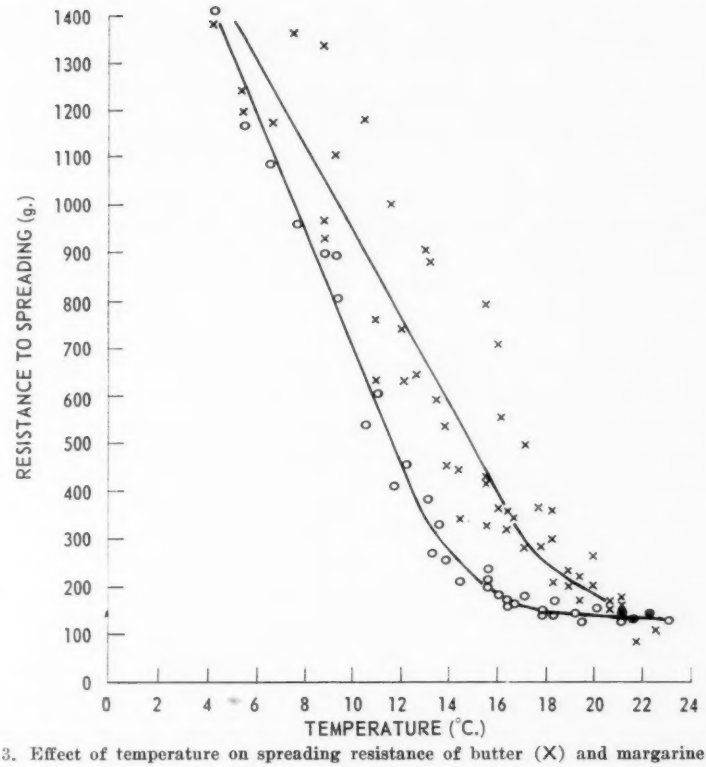

FIG. 3. Effect of temperature on spreading resistance of butter (X) and margarine (O).

with desirable spreadability (resistance of 260-530 g.) from the temperatures of the home refrigerator (4-7° C.) to the most usual room temperatures (21-24° C.).

The above specifications are only approximate, as they were not based on a consumer survey; however, until a broader consumer survey is made, these specifications should be an aid to assigning dimensional values to the effects of various treatments of fat, cream, or butter on the spreadability of the resulting butter. Consequently, much time could be saved in evaluating the significance of many steps in butter processing. The comparison of results between various laboratories could be facilitated if a standard instrument were to be adopted to measure spreadability.

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WHEY UTILIZATION. IV. AVAILABILITY OF WHEY NITROGEN FOR THE GROWTH OF *SACCHAROMYCES FRAGILIS*

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SUMMARY

Saccharomyces fragilis utilized approximately 50% of the ammonia nitrogen, 25% of the organic nitrogen, and 25% of the total nitrogen in whey, and all of the added ammonia nitrogen. The available nitrogen was found to be in the organic fraction soluble after heat- and acid-precipitation of the whey proteins.

Whey contains approximately 0.14% nitrogen. The principal nitrogenous components are the heat- and acid-precipitable lactalbumin and lactoglobulin, whereas the nonprotein nitrogen fraction consists of proteose-peptones, amino acids, and other substances. Orla-Jenson *et al.* (4) reported that the noncoagulable N in heat- and acid-treated whey was not utilized for growth by various strains of lactic acid bacteria, and whey so treated was used as a base for the addition of nitrogen sources in a study of the metabolism of these organisms. These authors also showed that although the bacteria used casein as a source of N for growth, they were unable to use lactalbumin N. Leviton and Whittier (2), on the other hand, reported that the nonheat-coagulable N fraction of whey was utilized for growth and riboflavin production by *Ashbyii gossypii.*

A study of the growth of *Saccharomyces fragilis* in whey showed that approximately 25% of the total whey nitrogen was used by the yeast (6). A further study, reported here, shows that the assimilable nitrogen was available only from the noncoagulable nitrogen fraction, and that the heat- and acid-precipitable proteins were not broken down by the actively growing yeast.

MATERIALS AND METHODS

S. fragilis, NRRL Y1109, was grown in a medium consisting of Cottage Cheese whey, 0.5% $(NH_4)_2SO_4$, 0.5% K_2HPO_4, and 0.1% yeast extract. The whey pH was about 4.6, but following the addition of the salts was approximately 5.6, which was in the range for optimal yeast growth. The temperature was maintained at $32 \pm 1°$, and the 500-ml. quantities of media in the propagators were aerated at 2 liters/minute. Aliquots of the growing yeast suspensions were removed for the determination of the nitrogen fractions. The yeast was sedimented immediately by centrifugation and supernatants treated as described in the test. Total nitrogen (TN) was determined by micro-Kjeldahl distillation following digestion by the Miller and Miller modification (3) of the Koch-McMeekin method (1). Ammonia nitrogen (AN)[1] was determined by micro-Kjeldahl distillation, and the organic nitrogen (ON) fraction was calculated as the difference between TN and AN.

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[1] Ammonia N was determined by the addition of alkali to undigested samples. Traces of volatile protein degradation products also may be distilled over in this determination.

Deproteinated whey was prepared from whey clarified by passage through a medium sintered glass filter. The pH was adjusted to 6.0 with a few drops of concentrated NaOH, and the whey heated in a boiling water bath for 10 min. (500- and 1,000-ml. quantities of whey were heated in the autoclave at 121° for 10 min.). After cooling, the pH of the whey was adjusted to 4.5, and the precipitated protein removed by centrifugation.

RESULTS

Yeasts were grown in whey media in the presence or absence of $(NH_4)_2SO_4$ as a source of inorganic nitrogen. The nitrogen composition of the media was determined after 4 hr. of growth. Results of the analyses are shown in Table 1.

TABLE 1

Course of nitrogen utilization during growth of *Saccharomyces fragilis* in whey medium, with and without added ammonium sulfate

Growth time	Whey medium					
	Minus $(NH_4)_2SO_4$			Plus $(NH_4)_2SO_4$		
	Total N	NH_4^+N	Organic N	Total N	NH_4^+N	Organic N
(hr.)	*(mg. N/100 ml.)*					
0	129	14	115	252	128	124
1	97	6	91	194	87	107
2	96	6	90	116	21	95
3	95	7	88	99	8	91
4	95	7	88	99	8	91
Yeast yield	14.7 g. dry wt/liter			25.9 g. dry wt/liter		

The total N of the medium without added $(NH_4)_2SO_4$ decreased to approximately 75% of its initial value within an hour after yeast growth began. Continued growth for several hours did not reduce further the nonutilizable N of the medium. When the yeasts were grown in the fully supplemented medium, the same pattern of N utilization was observed. The total N decreased to the same level as in the $(NH_4)_2SO_4$-free medium, and was not consumed further in spite of continued yeast growth. These results had been observed previously (6). The total N was separated into the ammonia N and organic N components. The whey medium, in the absence of $(NH_4)_2SO_4$, contains little AN, and the yeast used most of this nitrogen and about 25% of the ON fraction. The remainder of the ON was the nonutilizable residue. In the presence of added $(NH_4)_2SO_4$, the yeast readily used the added AN, although some ON disappeared simultaneously. However, when the ammonia nitrogen had been consumed, the nonutilizable ON residue remained.

The quantity of N in the whey available to the yeast was insufficient to support a good growth of yeast. In the presence of added nitrogen, the yeast yield obtained in 4 hr. of growth was almost twice as great as the yield in the absence of $(NH_4)_2SO_4$.

Whey organic nitrogen is composed of two fractions: (1) heat- and acid-precipitable N, and (2) noncoagulable, or soluble, N. When the whey was

submitted to the heat and acid treatment described in Methods, 52% of the ON was noncoagulable (Table 2). The presence of the medium supplements during the heat and acid treatment did not significantly affect the quantity of ON precipitated. However, it was noted that after the heat treatment, the pH of the whey alone remained at the initial value of 6.0, while the pH of the whey medium changed to 5.3.

TABLE 2

Effect of heat- and acid-deproteination on the nitrogenous components of whey alone and whey plus the medium salts

Treatment	Whey			Whey medium		
	Total N	NH_4^+ N	Organic N	Total N	NH_4^+ N	Organic N
			(mg. N/100 ml.)			
None	128	25	103	245	141	104
Heat and acid	74	20	54	198	139	59

Since the ON component of whey is composed of almost equal quantities of the noncoagulable and the heat- and acid-precipitable N fractions, it was of interest to determine which fraction was used by the yeast. Therefore, the growth of yeast in deproteinated whey medium was compared with growth in the regular whey medium. To 500-ml. quantities of deproteinated whey and untreated whey were added the salts and yeast extract necessary to formulate the media. After inoculation with *S. fragilis*, an aliquot was removed from each propagator, clarified by centrifugation, and the supernatants divided into two portions. One portion, labeled Pre-treatment, was refrigerated. The second portion was adjusted to pH 6.0, submerged in a boiling water bath for 10 min., and cooled. After the pH was adjusted to 4.5, any precipitate formed was removed by centrifugation. The supernatant solution, labeled Post-treatment, was refrigerated. The yeasts were grown in the two media for 3 hr., at which time all the lactose had disappeared and the maximum yield of yeast had been reached. Samples were again taken from the two propagators, and treated as described above. The analysis of the N components of the various samples is shown in Table 3. The initial heat- and acid-treatment of the whey removed approximately 53% of the whey organic nitrogen. During the 3-hr. growth period all of the ammonia N added to both the deproteinated and regular whey was

TABLE 3

Nitrogen disappearance in regular whey and heat- and acid-treated (deproteinated) whey media during growth of *Saccharomyces fragilis*

Growth time	Sample	Whey medium			Deproteinated whey medium		
		Total N	NH_4^+ N	Organic N	Total N	NH_4^+ N	Organic N
(hr.)				*(mg. N/100 ml.)*			
0	Pre-treatment	247	149	108	180	130	50
	Post-treatment	178	128	50	176	131	45
3	Pre-treatment	104	9	95	37	4	33
	Post-treatment	38	4	34	36	3	33

assimilated by the yeast, and in both media approximately the same quantity of nitrogen (15 mg/100 ml.) disappeared from the ON. Since, in the deproteinated whey medium the precipitable N had already been removed, the nitrogen utilized by the yeast originated in the remaining noncoagulable portion. In the regular whey medium, the ON utilized could have come from either the precipitable protein or the noncoagulable N component. The heat- and acid-treatment of the exhausted medium after 3 hr. of yeast growth revealed that the nitrogen used by the yeast in the whey medium also originated in the noncoagulable organic nitrogen fraction.

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VARIATION IN RECTAL TEMPERATURE, RESPIRATION RATE, AND PULSE RATE OF CATTLE AS RELATED TO VARIATION IN FOUR ENVIRONMENTAL VARIABLES[1]

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SUMMARY

To determine the relative importance of variations in air temperature, wind velocity, solar radiation, and vapor pressure in producing responses of cattle, a series of four 24-hr. studies in 1953 and five in 1954 were conducted with Brahman, Jersey-Brahman crossbred, Jersey, and Holstein females. Thirteen observations each of the rectal temperature, pulse rate, and respiration rate of each animal were recorded during the 24-hr. period, i.e., at the beginning of the test period and at intervals of 2 hr. Continuous records of solar radiation, air temperature, vapor pressure, and wind velocity were obtained. Though the primary object was not a breed comparison, the data conform to the findings of previous studies with respect to the relative heat tolerance of the groups studied, the order of heat tolerance being Brahman, most heat-tolerant, followed by ½ Jersey–½ Brahman, ¾ Jersey–¼ Brahman, Jersey, and Holstein.

The data were studied by multiple regression methods, using as independent variables the four environmental variables and as dependent variables those measured on the cattle. The results indicate that under conditions similar to those prevailing during these studies, almost as satisfactory explanation of variation in rectal temperature, respiration rate, and pulse rate can be accomplished with air temperature as the only independent variable as with all four environmental variables included. The percentages of variance due to the regression of the measured physiological responses on all the independent variables did not differ much from those considering air temperature alone.

From a practical standpoint, it may be concluded that air temperature is the most important environmental variable with respect to summer weather stress in cattle. Management procedures to combat summer weather stress should emphasize efforts to provide as low air temperatures as economically feasible. This does not mean, however, that no effort should be devoted to the provision of shade for cattle.

Several investigators have reported the results of studies of physiological responses of cattle to ambient conditions of air temperature and humidity, in many cases closely controlled, in chambers designed for this purpose. These reports constitute valuable contributions to our understanding of the fundamental processes involved in heat regulation in the animal body. McDowell (1) has presented an excellent review of these studies. The present paper presents the results of experiments designed to study the same phenomena under natural conditions, with environmental variables continuously recorded to permit sta-

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[1] The data on which this paper is based were collected in studies conducted as part of a project supported cooperatively by the Texas Agricultural Experiment Station and the Dairy Cattle Research Branch, Agricultural Research Service, United States Department of Agriculture.

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tistical control rather than the physical control attained in psychrometric chambers. Such studies seem to be logical intermediate steps in the process of applying the fundamental knowledge acquired from control chamber studies to practical problems in breeding and management.

MATERIALS AND METHODS

During the summers of 1953 and 1954 data were collected during nine 24-hr. periods from nonlactating Brahman, Jersey-Brahman crossbred, Jersey, and Holstein females. In the four 1953 test periods four Brahmans (B.), ten ½ Jersey–½ Brahman (½ J.–½ B.), two ¾ Jersey–¼ Brahman (¾ J.–¼ B.), ten Jerseys (J.), and ten Holsteins (H.) were used. In the five 1954 tests the numbers of Jerseys, Holsteins, and halfbreds were the same as in 1953, but no Brahmans were used and four ¾ Jersey–¼ Brahman were available. The age ranges in 1953 were: Brahman, 4 to 5 yr.; ¾ Jersey–¼ Brahman, 3 to 10 mo.; ½ Jersey–½ Brahman, 6 to 19 mo.; Jersey, 4 to 18 mo., and Holstein, 6 to 18 mo. In 1954 they were: ¾ Jersey–¼ Brahman, 6 to 15 mo.; ½ Jersey–½ Brahman, 5 to 19 mo.; Jersey, 6 to 20 mo., and Holstein, 6 to 21 mo. These age ranges were as narrow as could be attained with the animals available for study.

The animals were tied in an open lot free of obstruction to solar radiation and air movement. Each test began at 5 P.M. on one day and ended at 5 P.M. on the following day. Periods of from one to three days were allowed between tests to permit the animals to recover from the stress of the tests. Records were taken initially and at 2-hr. intervals of rectal temperature, respiration rate, and pulse rate of all animals except the Brahmans. These had been ranch-raised, were trained to halter, and were tractable under most circumstances, but would become excited when rectal temperature was taken or when an artery (usually the saphenous) was palpated to determine pulse rate. Because of the extreme inconvenience involved in collecting Brahman rectal temperature and pulse rate data, and the fact that the animals' excitement would very likely render the data misleading, only respiration rate (taken by counting flank movements during a timed interval) was recorded for the Brahmans. Solar radiation, air temperature, relative humidity (used to calculate vapor pressure), and wind velocity were recorded continuously by means of a pyrheliometer, a hygrothermograph, and a thermal anemometer, respectively. The pyrheliometer, an instrument with which many readers may not be familiar, measures the total radiation intensity on a horizontal surface, including the direct solar radiation and that reflected from the whole hemisphere of the sky.

The data were subjected to analysis of variance to assess various sources of variation. To compare the environmental variables with respect to their apparent relative contributions to the variation of the physiological measurements taken on the animals, multiple regression analyses were conducted using the environmental variables as independent variables and the physiological measurements as dependent variables. For the environmental variables the values used in the analysis were the averages for the hour preceding the time at which corresponding values for the physiological measurements were recorded,

TABLE 1
Ranges of environmental variables

	Wind velocity (miles per hour)		Solar radiation (calories per sq. cm. per minute)	Air temperature (Degrees Fahrenheit)		Vapor pressure (mm. Hg)	
	Day	Night		Day	Night	Day	Night
1953	2.0 to 7.0	0.6 to 6.3	0.02 to 1.50	76° to 101°	73° to 89°	16.2 to 24.3	18.0 to 25.1
1954	2.4 to 12.5	1.1 to 7.7	0.05 to 1.29	70° to 99°	69° to 86°	12.2 to 23.5	13.1 to 22.8

since it is known that effects on the animals of changes in the ambient conditions are not instantaneous but gradual and cumulative. The data were divided into two parts for these analyses, viz., sunlight data and nonsunlight data, since only three of the four recorded environmental variables were considered during nonsunlight hours, solar radiation being effectively nonvariable from sunset to sunrise.

RESULTS AND DISCUSSION

The observed ranges of the four environmental variables during the test periods in the 2 yr. are shown in Table 1, to convey a general impression of the conditions to which the animals were subjected. Mention should be made of one striking difference between summer conditions at College Station, Texas, and those in other subtropical and tropical areas where similar research has been conducted. Extremely high air temperature seldom occurs simultaneously with extremely high humidity. This undoubtedly accounts for the fact that vapor pressure in the present data does not appear to have as much influence on animal responses as it has in other locations where similar studies have been conducted.

The over-all averages for the three physiological measurements are given in Table 2. No significance can be attached to the ranking of these group averages because of the small numbers of animals and some confounding of age and breeding, the average ages of the groups of animals available for study being quite different from one another. Adjustment of the physiological measurements for age differences would have been quite unreliable because of effects

TABLE 2
Breeding group averages for physiological measurements

Breeding group	Rectal temperature (° F.)		Respiration rate (per minute)		Pulse rate (per minute)	
	1953	1954	1953	1954	1953	1954
B.	.....	.....	30.9	.....	.....	.....
½ J.–½ B.	102.2	101.6	45.9	36.6	71.4	65.7
¾ J.–¼ B.	102.3	101.7	48.3	43.5	84.6	58.8
J.	102.3	101.6	65.7	51.9	80.4	63.0
H.	103.1	102.0	82.2	60.0	76.2	59.4

TABLE 3

Analyses of variance

Source of variation	Degrees of freedom	Mean squares		
		Rectal temperature	Respiration rate	Pulse rate
1953				
Total	1663 (1871)[a]			
Days	3	25.9**	1394.0	231.3**
Breeding groups within day	12 (16)	23.3**	3970.9**	267.4**
Animals within day and breeding group	112 (1214)	6.5**	141,3**	112.4**
Measurements on the same animal within day and breeding group	1536 (1728)	1.1	86.4	9.7
1954				
Total	2209			
Days	4	19.5**	1787.8**	288.5**
Breeding groups within day	15	6.7**	1429.7**	107.6**
Animals within day and breeding group	150	2.7**	98.9*	82.5**
Measurements on the same animal within day and breeding group	2040	0.8	80.5	9.3

*, ** Conventional significance designation, used throughout this paper, one asterisk indicating statistical significance between the 0.05 and 0.01 probability levels and two asterisks indicating statistical significance at a level of probability of 0.01 or less.

[a] Numbers in parentheses are degrees of freedom for the analysis of respiration rate in 1953.

of sampling resulting from the small number of each age and breeding used, the above-mentioned confounding of age and breeding in the group comparisons, and the inevitable confounding of age and individuality within groups.

Analyses of variance of the three physiological measurements are shown in Table 3. Because of the effects of sampling due to the small number of animals used, and the previously mentioned confounding of age effects, the differences among breeding groups, as well as differences among animals within groups reflected by the analyses, are not entirely the result of group differences in breeding or differences in individuality within groups. If reliable age adjustments could be accomplished, it appears that the breeding groups would be ranked in order of decreasing heat tolerance approximately as would be expected from previous studies, viz., Brahman, ½ Jersey–½ Brahman, ¾ Jersey–¼ Brahman, Jersey, and Holstein.

Since a breed comparison was not the primary purpose of the work reported here, the significant day, group, and animal differences reflected by the analyses of variance served only to dictate the performing of the multiple regression calculations on a within-animal-and-day basis separately for each breeding group.

Total, direct, and indirect contributions of each environmental variable to the squared multiple correlation coefficient are presented in Tables 4, 5, and 6. The direct contribution is that which the independent variable in question would make if the other independent variables were held constant, and the indirect contribution is that which the variable in question makes through its correlation with the other independent variables considered in the multiple

TABLE 4

Contributions of each independent variable to R^2
(Rectal temperature)

	½ J.–½ B.			¾ J.–¼ B.			J.			H.		
	Total	Direct	Indirect	Total	Direct	Indirect	Total	Direct	Indirect	Total	Direct	Indirect
1953												
Wind velocity	0.0475	0.0388	0.0087	0.0057	0.0012	0.0045	0.0234	0.0098	0.0136	0.0284	0.0139	0.0145
Solar radiation	−0.0598	+0.0790	−0.1388	+0.0231	0.0029	0.0202	0.0018	0.0000	0.0017	−0.0538	0.0161	−0.0699
Air temperature	0.3811	0.5112	−0.1301	0.3730	0.3516	0.0214	0.4940	0.4706	0.0234	0.6569	0.7303	−0.0470
Vapor pressure	−0.0289	0.0049	0.0250	−0.0025	0.0000	−0.0025	0.0090	0.0003	0.0087	−0.0011	0.0000	−0.0011
	$R^2 = 0.3401$			$R^2 = 0.3993$			$R^2 = 0.5281$			$R^2 = 0.6304$		
1954												
Wind velocity	0.0176	0.0022	0.0154	0.0970	0.0375	0.0595	0.0062	0.0003	0.0059	0.0211	0.0028	0.0183
Solar radiation	0.0613	0.0135	0.0478	0.1165	0.0412	0.0753	0.1169	0.0402	0.0767	0.1051	0.0322	0.0729
Air temperature	0.5794	0.6000	−0.0206	0.5234	0.4310	0.1014	0.5548	0.5112	0.0436	0.6244	0.5579	0.9665
Vapor pressure	−0.0595	0.0289	−0.8840	−0.0273	0.0044	−0.0317	−0.0339	0.0073	−0.0412	−0.0223	0.0024	−0.0247
	$R^2 = 0.5987$			$R^2 = 0.7186$			$R^2 = 0.6440$			$R^2 = 0.7283$		

TABLE 5

Contributions of each independent variable to R^2
(Respiration rate)

	½ J.–½ B.			¾ J.–¼ B.			J.			H.		
	Total	Direct	Indirect	Total	Direct	Indirect	Total	Direct	Indirect	Total	Direct	Indirect
1953												
Wind velocity	−0.0048	0.0003	−0.0051	−0.0080	0.0023	−0.0103	−0.0127	0.0108	−0.0235	−0.0112	0.0112	−0.0224
Solar radiation	0.2031	0.0930	0.1101	0.1326	0.0416	0.0910	0.1761	0.0697	0.1064	0.1137	0.0331	0.0806
Air temperature	0.4307	0.4096	0.0211	0.6043	0.6273	−0.0230	0.5587	0.5670	−0.0083	0.6258	0.6691	−0.0433
Vapor pressure	−0.0558	0.0454	−0.1012	−0.0748	0.0424	−0.1127	−0.0715	0.0475	−0.1190	−0.0777	0.0424	−0.1201
	$R^2 = 0.5732$			$R^2 = 0.6541$			$R^2 = 0.6506$			$R^2 = 0.6506$		
1954												
Wind velocity	−0.0091	0.0072	−0.0163	0.0118	0.0028	0.0920	−0.0093	0.0143	−0.0241	−0.0096	0.0035	−0.0131
Solar radiation	0.3910	0.2973	0.0937	0.4328	0.3487	0.0841	0.4727	0.3769	0.0958	0.4114	0.2993	0.1121
Air temperature	0.1845	0.1269	0.0576	0.1487	0.0687	0.0800	0.1786	0.1047	0.0739	0.2443	0.1647	0.0796
Vapor pressure	−0.0167	0.0216	−0.0383	−0.0102	0.0037	−0.0139	−0.0123	0.0573	−0.0696	−0.0210	0.0130	−0.0340
	$R^2 = 0.5496$			$R^2 = 0.5830$			$R^2 = 0.6297$			$R^2 = 0.6252$		

TABLE 6

Contributions of each independent variable to R^2
(Pulse rate)

	½ J.–½ B.			¾ J.–¼ B.			J.			H.		
	Total	Direct	Indirect	Total	Direct	Indirect	Total	Direct	Indirect	Total	Direct	Indirect
1953												
Wind velocity	0.0732	0.0645	0.0087	0.0579	0.0328	0.0251	0.0336	0.0180	0.0156	0.0847	0.0762	0.0085
Solar radiation	−0.0359	0.0335	−0.0694	0.0740	0.0225	0.0515	0.0361	0.0079	0.0282	−0.0291	0.0216	0.00507
Air temperature	0.1725	0.2591	−0.0866	0.2396	0.2470	−0.0074	0.2280	0.2070	−0.0210	0.1389	0.1884	−0.0495
Vapor pressure	−0.0298	0.0240	−0.0538	−0.0337	0.0416	−0.0753	−0.0141	0.0025	−0.0166	−0.0213	0.0128	−0.0341
	$R^2 = 0.1796$			$R^2 = 0.3378$			$R^2 = 0.2836$			$R^2 = 0.1734$		
1954												
Wind velocity	0.0597	0.0340	0.0257	0.0985	0.0661	0.0324	0.0893	0.0615	0.0278	0.0622	0.0393	0.0229
Solar radiation	0.0210	0.0067	0.0143	0.0461	0.0220	0.0241	0.0084	0.0021	0.0063	−0.0068	0.0041	0.0109
Air temperature	0.1203	0.0840	0.0363	0.1102	0.0647	0.0455	0.0723	0.0317	0.0406	0.0887	0.0763	0.0124
Vapor pressure	−0.0009	0.0000	0.0009	−0.0056	+0.0008	−0.0064	0.0425	0.0194	0.0230	−0.0030	0.0002	−0.0032
	$R^2 = 0.2001$			$R^2 = 0.2491$			$R^2 = 0.2147$			$R^2 = 0.1411$		

correlation system under study. The partial regression coefficients were so computed that they gave the amount of change in the dependent variables per degree change in air temperature, per 0.1 cal. change in solar radiation and per 0.5 mm. Hg vapor pressure. The numerical ranges of the independent variables in these units are more nearly equal than in the actual units of measurement.

To facilitate comparison of the environmental variables with respect to their relative importance, Tables 7 and 8 were prepared. The values in these tables were obtained by dividing each partial regression of a dependent variable on an independent variable by the partial regression of that dependent variable on air temperature, thus making regression on air temperature the standard of comparison. A negative value indicates that the effect of the independent variable in question was opposite to that of air temperature.

It is apparent that air temperature is the most important of the four environmental variables studied with respect to accounting for variation in the physiological measurements. In fact, because the correlation of air temperature with each of the physiological measurements is relatively large, incorporation of the other environmental variables into a multiple correlation system along with air temperature permits explanation of very little more of the variation in the physiological measurements than does the use of air temperature alone. This is true of any multiple correlation system in which correlations among the independent variables are relatively large, which is the case in the present data, the correlations between air temperature and solar radiation, for example, being 0.61 and 0.54 in 1953 and 1954, respectively.

A detailed tabular presentation of all 304 product-moment correlations computed would serve little purpose. However, the fact that air temperature alone provides almost as satisfactory (or, in most cases, just as satisfactory, statistically speaking) explanation of the variation in the physiological measurements and prediction of them as does the use of all the environmental variables can be demonstrated by comparing the multiple correlation coefficient with the product-moment correlation between air temperature and the physiological measurement in question. This comparison is presented in Table 9. In most cases the multiple correlation (R) is only slightly larger than the corresponding product-moment correlation (r) involving air temperature.

Some of the values in Tables 4-8 are contrary to expectation, in that they are negative when a priori reasoning would indicate that they should be positive. Such values are relatively few, and interpretations must be based on the general pattern. Several of the small negative values are undoubtedly the result of sampling and are estimates of zero. Vapor pressure appears inconsistently to have some influence, but air temperature and solar radiation are the most consistently influential variables.

Solar radiation appears, in general, to follow air temperature in relative importance among the four environmental variables considered here and, in some cases, can explain an appreciable portion of the variation in physiological measurements in addition to that provided by air temperature alone. This is

TABLE 7

Apparent relative effectiveness of the four environment variables
(Sunlight hours)
Dependent variables

Independent variables	Rectal temperature				Respiration rate					Pulse rate			
	½ J.–½ B.	¾ J.–¼ B.	J.	H.	B.	½ J.–½ B.	¾ J.–¼ B.	J.	H.	½ J.–½ B.	¾ J.–¼ B.	J.	H.
1953													
Wind velocity	0.34	0.07	0.18	0.18	0.74	−0.05	−0.08	−0.17	−0.16	0.62	0.45	0.37	0.80
Solar radiation	−0.61	0.14	0.09	−0.23	0.27	0.75	0.41	0.55	0.35	−0.56	0.34	0.31	0.53
Air temperature	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vapor pressure	0.22	0.02	−0.06	0.004	0.84	0.78	0.61	0.68	0.59	0.71	0.97	0.26	0.61
1954													
Wind velocity	0.05	0.28	0.02	0.07	.....	−0.22	0.19	−0.36	−0.14	0.50	0.94	1.12	0.67
Solar radiation	0.28	0.58	0.53	0.45	.....	2.91	4.27	3.60	2.56	0.53	1.09	0.49	−0.44
Air temperature	1.00	1.00	1.00	1.00	.....	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vapor pressure	0.46	0.20	0.25	0.14	.....	0.87	0.49	0.50	0.60	0.03	0.24	−0.16	0.12

TABLE 8

Apparent relative effectiveness of the four environment variables
(Nonsunlight hours)
Dependent variables

Independent variables	Rectal temperature				Respiration rate					Pulse rate			
	½ B.–½ J.	¾ J.–¼ B.	J.	H.	B.	½ J.–½ B.	¾ J.–¼ B.	J.	H.	½ J.–½ B.	¾ J.–¼ B.	J.	H.
1953													
Wind velocity	−0.38	−0.16	−0.13	−0.27	−0.54	−0.64	−0.03	−0.36	−0.69	−0.25	−0.36	−0.67	0.30
Air temperature	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vapor pressure	−0.74	0.25	0.17	−0.96	−0.62	−0.95	0.56	−0.45	−0.92	0.35	−0.26	−0.23	0.43
1954													
Wind velocity	−0.50	−0.05	0.14	0.07	.....	−0.12	−0.04	−0.28	2.24	−0.18	0.18	−0.09	−0.25
Air temperature	1.00	1.00	1.00	1.00	.....	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Vapor pressure	−0.26	0.20	−0.39	−0.34	.....	−0.09	0.04	0.19	−0.27	0.37	0.16	0.18	−1.06

TABLE 9

Product-moment correlations (r) involving air temperature and multiple correlations (R) obtained with all environmental variables included

	Rectal temperature		Respiration rate		Pulse rate	
	r	R	r	R	r	R
1953						
Sunlight						
B.	.....	.....	0.356**	0.445**	.....	.....
½ J.–½ B.	0.533**	0.583**	0.673**	0.757**	0.399**	0.424**
¾ J.–¼ B.	0.629**	0.632**	0.763**	0.809**	0.482**	0.581**
J.	0.720**	0.727**	0.742**	0.807**	0.501**	0.660**
H.	0.783**	0.794**	0.765**	0.807**	0.320**	0.416**
Nonsunlight						
B.	.....	.....	0.429**	0.483**	.....	.....
½ J.–½ B.	0.531**	0.563**	0.476**	0.594**	0.444**	0.458**
¾ J.–¼ B.	0.616**	0.620**	0.711**	0.733**	0.1200	0.208
J.	0.424**	0.430**	0.538**	0.566**	0.189*	0.256*
H.	0.491**	0.529**	0.336**	0.438**	0.351**	0.354**
1954						
Sunlight						
½ J.–½ B.	0.744**	0.748**	0.518**	0.741**	0.415**	0.447**
¾ J.–¼ B.	0.811**	0.848**	0.567**	0.764**	0.433**	0.499**
J.	0.776**	0.802**	0.552**	0.794**	0.406**	0.463**
H.	0.836**	0.853**	0.602**	0.791**	0.321**	0.376**
Nonsunlight						
½ J.–½ B.	0.530**	0.534**	0.360**	0.366**	0.383**	0.403**
¾ J.–¼ B.	0.683**	0.688**	0.409**	0.409**	0.593**	0.615**
J.	0.535**	0.541**	0.110	0.137	0.546**	0.551**
H.	0.531**	0.537**	0.115	0.165*	0.314**	0.355**

particularly true in the 1954 data on respiration rate, where the values in Table 7 indicate that solar radiation was a more influential variable than air temperature. This accounts for the greater differences seen in the r vs. R comparison for 1954 respiration rate in Table 9. There are indications in the data to the effect that at extremely high air temperatures, changes in solar radiation are less effective in producing variation in physiological measurements than at lower air temperatures. This might be construed to imply that shade is of little value when air temperature is extremely high. However, it is obvious that cattle in the direct sunlight at any air temperature would be bearing a greater heat load than cattle in the shade at the same air temperature. Apparently, the high air temperatures stress the cattle nearly to the limit of their ability to reflect stress in their rectal temperature, respiration rate, and pulse rate.

REFERENCE

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A STUDY OF THE INFLUENCE OF SOLAR RADIATION ON PHYSIOLOGICAL RESPONSES OF DAIRY CATTLE[1]

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SUMMARY

Effects of the subtropical climate of central Texas on the heat-regulatory ability of dairy heifers were studied in 1955 and 1956. Physiological responses of the heifers—body temperature, pulse rate, and respiration rate—to environmental effects—air temperature, solar radiation, vapor pressure, and wind velocity—were studied. The effects of solar radiation were of primary interest.

Solar radiation had a direct effect on body temperature when air temperatures were close to the range of thermo-neutrality (below 90° F.). In higher air temperatures (above 90° F.), solar radiation had little effect on the changes in body temperatures. Respiration rate, the most consistent physiological response studied, was affected more by solar radiation than by the other weather influences. Large differences in respiration rate due to genetic origin were also observed. It could not be demonstrated satisfactorily that pulse rates were influenced by any of the environmental variables.

Body temperature data and respiration rate data taken on animals in the absence of direct solar radiation were almost as reliable indicators of differences in heat regulatory ability as the same measurements taken on animals in the presence of direct solar radiation.

The physiological responses of purebred European cattle, of purebred Zebu or Brahman cattle, and of crossbreds between them, to variations in air temperature, humidity, wind velocity, and solar radiation, largely determine the degree of adaptability of these animals to tropical and subtropical climates. The effects of air temperature and humidity on dairy cattle heat tolerance have been investigated by Gaalaas (4), Kibler and Brody (7, 8, 9, 10), Riek and Lee (13), Johnston and Frye (6), Cartwright (2), and Shrode *et al.* (17). A summary of their work as presented by McDowell (11) indicates that air temperature and its interaction with humidity are very important influences on cattle adaptation to these regions. Other than the multiple effects of air temperature on the physiological responses of cattle, resistance to direct solar radiation has been the most frequently pursued subject related to cattle heat tolerance. Most of the investigations have been devoted to research on solar radiation resistance resulting from surface area and various other characteristics of the animal hide. These studies were based on the logical assumption

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that solar radiation is a major contributing factor to heat distress, although little has been done to establish this as a fact.

The present study was designed to determine the relative importance of solar radiation as a factor contributing to heat distress in Jersey, Holstein, and Brahman-Jersey crossbred heifers in the central Texas region.

MATERIALS AND METHODS

The data were collected in two different years. In the summer of 1955 18 dairy heifers—six Jersey (J.) heifers, six ½ Jersey–½ Brahman (½ J.–½ B.) heifers, and six ¾ Jersey–¼ Brahman (¾ J.–¼ B.) heifers—ranging in age from 7 to 27 mo., were placed on experiment. Because of a lack of young half-bred females in the summer of 1956, six Holstein heifers within the same age limits were used to replace the ½ Jersey–½ Brahman group. The individual heifers were exposed to sun and shade over a test period of nine alternate days. At the end of the experimental period, each heifer within each genetically defined group had been exposed to six days of sunlight and three days of shade. To obtain the maximum in measurement accuracy and the maximum amount of information, the heifers were removed from feed and water and were tied in their randomly assigned positions before sunrise. The shaded animals were secured under well-ventilated sun shades erected over sod, while the remaining animals were tied in the open where they would receive the maximum amount of solar radiation. During the day, the shaded heifers were moved only as much as was necessary to insure that they remained within the shade provided for them.

Beginning at 6 A.M. and continuing hourly until 5 P.M., measurements were taken on each animal. Body temperatures to the nearest one-tenth of a degree were obtained with an ordinary 5-in. veterinary thermometer. Pulse rates based on a 10-sec. palpation of the saphenous artery and visual respiratory counts of 20-sec. duration were timed with a stop watch. At the end of each test period, the heifers were freed and were allowed free access to water and feed. One day of rest was allowed between tests.

Hourly averages of air temperature, relative humidity, solar radiation, and wind velocity were computed from continuous records during the two summer test periods. Air temperatures and relative humidity were recorded continuously by a hygrothermograph. Vapor pressure was calculated from these records for use in the analyses. Using a pyrheliometer and a thermal anemometer, solar radiation and wind velocity were recorded continuously on a Brown Electronik recorder. The pyrheliometer measures the total radiation intensity on a horizontal surface, including the direct solar radiation and that reflected from the whole hemisphere of the sky. This is the radiation from which shaded animals would, to a great extent, be protected. They would receive radiation reflected from their surroundings and from the ground, and their total radiation load would probably be about half that of nonshaded animals, according to measurements made by Ittner and Kelly (5) under similar shades.

Separate analyses were made of each year's data. An analysis of variance

was used to determine the significance of differences in physiological responses among the genetic groups within a location (sun or shade). A regrouping of the data then permitted an investigation of the differences in physiological responses of animals between sun and shade locations within one genetic group.

A multiple regression analysis, in which the environmental measurements were used as independent, causative variables and the animal body measurements were used as the dependent variables, was conducted to illustrate the relative importance of the environmental variables as causes of heat distress. The data were divided into groups of measurements taken in air temperatures above 90° F. and those taken below 90° F., and statistical estimates were calculated separately on shade measurements and on sun measurements on a within-animal and within-genetic group basis.

RESULTS

The 1955 complete analysis of variance of body temperature indicated that two of the three main effects were the major causes of variations in rectal temperature. As was expected, daily changes in the environment and the animals' physiology were reflected in the analysis. More important, locations differed in their effect on the heifers. A within-genetic-group analysis of location differences in body temperature, shown in Table 1, indicates that the three

TABLE 1

Analysis of variance of body temperature to partition variation due to location differences within genetic groups (1955)

Sources of variation	Degrees of freedom	Mean square
Locations within genetic groups	(3)	136.48**
Jersey	1	189.34**
½ Jersey–½ Brahman	1	66.74**
¾ Jersey–¼ Brahman	1	153.36**
Error	124	7.43

* Significant at the .05 level of probability.
** Significant at the .01 level of probability.
(These conventional significance designations are used throughout this paper.)

genetic groups displayed different responses in body temperature due to the presence or absence of direct solar radiation.

In 1956, locations, genetic groups, days, and the second-order interactions involving days were significant. The analyses of Tables 2 and 3 show that the Holstein and three-quarter bred heifers were affected by variations in locations, whereas the Jersey heifers were not, and that within a fixed environment the three groups differed significantly in body temperature.

The data collected on pulse rates in both years substantiated the observation of Seath and Miller (16), viz., that this physiological factor was the least desirable for appraising heat tolerance. The only consistently significant main effect observed in the analyses was that of days.

Uniformity of results characterized the analysis of respiration rates for 1955 and 1956. In the complete analyses of both years, location, genetic group,

TABLE 2

Analysis of variance of body temperature to partition variation due to location differences within genetic groups (1956)

Sources of variation	Degrees of freedom	Mean square
Locations within genetic groups	(3)	243.17**
Holstein	1	345.96*
Jersey	1	185.90
¾ Jersey–¼ Brahman	1	197.64*
Locations × days within genetic groups	(24)	37.71
Holstein	8	49.29
Jersey	8	35.39
¾ Jersey–¼ Brahman	8	28.45

TABLE 3

Analysis of variance of body temperature to partition variation due to differences within locations (1956)

Sources of variation	Degrees of freedom	Mean square
Genetic groups within locations	(4)	675.00**
Sun	2	989.47**
Shade	2	360.53**
Genetic groups × days within locations	(32)	32.05
Sun	16	29.03
Shade	16	35.07

and day effects were highly significant, whereas differences in no other classification exceeded the .05 level of significance. The differences among the three genetic groups within locations and the difference between the two locations within genetic groups were highly significant in each year.

The differences observed in the physiological responses suggested that a multiple regression study would be useful to summarize the degree of relationship of all known, important influences on a dependent animal response. The data were arbitrarily divided into those taken when air temperature was above 90° F. and those taken when air temperature was below 90° F., to determine if solar radiation differs in its effectiveness at high air temperatures from that which it exhibits at lower air temperatures.

Among the multiple correlations involving body temperature shown in Table 4, many are significant. In the 1955 data from the sun location, the correlations from the high-air-temperature data were larger in all of the groups but one. In two genetic groups in the 1956 trial, the trends of the 1955 data were contradicted. In the low range of temperatures, the Holstein heifers had a higher correlation coefficient resulting from the measurements taken in the shade than they did from the measurements taken in the sun. The ¾ Jersey–¼ Brahman heifers showed the same reversal in the higher temperature range. Another trend in these analyses was a decrease in the effect of the environmental influences on body temperature, with an increase in the fraction of Brahman inheritance. A difference in air temperature ranges (90° F. − and 90° F. +) was indicated by the generally larger size of the multiple correlation coefficients in the high-air-temperature data.

TABLE 4
Multiple correlation coefficients of environmental influences and body temperature

	Sun $R_{t\cdot asvw}$[a]	Shade $R_{t\cdot avw}$		Sun $R_{t\cdot asvw}$	Shade $R_{t\cdot avw}$
1955			1956		
90° F.–			90° F.–		
J.	0.457**	0.417**	H.	0.582**	0.646**
½ J.–½ B.	0.217*	0.315*	J.	0.493**	0.100
¾ J.–¼ B.	0.333**	.206	¾ J.–¼ B.	0.331**	0.12
	192 d.f.	95 d.f.		216 d.f.	107 d.f.
90° F.+			90° F.+		
J.	0.56**	0.243	H.	0.683**	0.581**
½ J.–½ B.	0.49**	0.438**	J.	0.719**	0.429**
¾ J.–¼ B.	0.40**	0.339**	¾ J.–¼ B.	0.387**	0.444**
	224 d.f.	111 d.f.		200 d.f.	99 d.f.

[a] The variables are indicated by the following symbols: a, air temperature; s, solar radiation; v, vapor pressure; w, wind velocity; p, pulse rate; r, respiration rate; and t, body temperature.

The analyses involving pulse rate failed to yield uniform results (Table 5). The statistically significant correlation coefficients in any group were not repeated within the same year at a different temperature range, nor were they repeated between years at the same range of temperatures. The only pattern noticeable is the higher degree of relationship obtained from the data taken at lower air temperatures.

The results obtained with respiration rate (Table 6) were very consistent. With the exception of one shade value in the high-air-temperature data in 1955, all the values were highly significant. In every genetic group, there was an increase in the size of the correlation coefficients when measurements were taken in the sun. When measurements were taken in the sun or in the higher range of air temperatures, there was a tendency for the multiple correlation coefficients to decrease with an increase in Brahman genes. The multiple correlation coefficients were smaller from the high-air-temperature data.

In these experiments, solar radiation was the only environmental variable over which any control was exercised. It was assumed that the shaded heifers

TABLE 5
Multiple correlation coefficients of environmental influences and pulse rate

	Sun $R_{p\cdot asvw}$	Shade $R_{p\cdot avw}$		Sun $R_{p\cdot asvw}$	Shade $R_{p\cdot avw}$
1955			1956		
90° F.–			90° F.–		
J.	0.328**	0.218	H.	0.602**	0.598**
½ J.–½ B.	0.350**	0.256	J.	0.579**	0.450**
¾ J.–¼ B.	0.338**	0.417**	¾ J.–¼ B.	0.653**	0.378**
	192 d.f.	95 d.f.		216 d.f.	107 d.f.
90° F.+			90° F.+		
J.	0.368**	0.318**	H.	0.199	0.249
½ J.–½ B.	0.241*	0.072	J.	0.194	0.146
¾ J.–¼ B.	0.319**	0.352**	¾ J.–¼ B.	0.221*	0.224
	224 d.f.	111 d.f.		200 d.f.	99 d.f.

TABLE 6

Multiple correlation coefficients of environmental influences and respiration rate

	Sun $R_{r.asvw}$	Shade $R_{r.avw}$		Sun $R_{r.asvw}$	Shade $R_{r.avw}$
1955			1956		
90° F.–			90° F.–		
J.	0.709**	0.521**	H.	0.733**	0.562**
½ J.–½ B.	0.720**	0.628**	J.	0.707**	0.590**
¾ J.–¼ B.	0.634**	0.340**	¾ J.–¼ B.	0.646**	0.606**
	192 d.f.	95 d.f.		216d.f.	107 d.f.
90° F.+			90° F.+		
J.	0.739**	0.441**	H.	0.702**	0.498**
½ J.–½ B.	0.576**	0.352**	J.	0.692**	0.489**
¾ J.–¼ B.	0.559**	0.120	¾ J.–¼ B.	0.531**	0.379**
	224 d.f.	111 d.f.		200 d.f.	99 d.f.

were subjected to the same uncontrolled variations in environment as were the other heifers. To obtain this effect, the animals were confined to a small area where there were no noticeable restrictions to any environmental effects other than solar radiation. In the analysis of variance, the uncontrolled weather conditions were combined under the heading of days.

Since weather conditions, other than the presence or absence of direct solar radiation determined by the location of the animal with reference to a sun shade, are important, consideration of the general conditions of the two trials is necessary.

In 1955, when the research was initiated, moderate summer weather for the area was recorded. The highest daily air temperatures averaged less than 100° F. In 1956, more nearly typical summer weather conditions prevailed, and the highest daily air temperature recorded was 105° F. During both years drouth conditions existed, but on the eighth day of the 1956 trial, overcast skies and light rains relieved the heat. In neither of the trials were any unusually high wind velocities observed.

Of the three physiological responses studied, body temperature is the least variable and is expected to have the smallest population variance. This was demonstrated amply in the complete analyses of variance, where the error terms which estimate population variance were small. This low initial variance makes body temperature an excellent measurement for studying environmental effects on the heat-regulatory ability of dairy cattle.

Exposure to solar radiation affects body temperature fluctuations, as indicated by the highly significant location effect. The locations-within-genetic-groups analysis in Table 1 emphasizes this. Of particular interest are the crossbred heifers. In 1955, the component of variance for the group with the highest frequency of Brahman genes is smaller than the components for the other groups. Because the components for the half-bred heifers are significant, as are the other components, this may not be important, except that in five of the six within-genetic-groups analyses this same mean square was nearly equal to or smaller than the other components. The present study confirms the previously demonstrated phenomenon of the first generation of a cross between

European and Brahman cattle, being less affected by extremes in solar radiation than purebred cattle of European origin (Jersey and Holstein), or than the generation resulting from a backcross to the European cattle (¾ Jersey–¼ Brahman).

The failure of differences among genetic groups to be statistically significant in the 1955 data is difficult to explain. Because the differences between groups within locations were not significant, one logical assumption is that the less variable air temperatures observed in 1955 had less effect on body temperature than did the more variable air temperatures in 1956. If this is true, the differences in groups are minimized by the small population variance attributed to the species.

The most notable feature of the analyses of the pulse rate data was the inconsistency of results. The data obtained in 1955 behaved very much like the data obtained on body temperature and respiration rate. The three main effects indicated differences due to solar radiation, genetic origin, and daily variations in the environment. On the basis of the 1955 results, the data from 1956 showed many deviations from expectation, since only days and the second-order interaction were significant. If the data presented were an accurate representation of the behavior of pulse rate, then failure of the results to be repeated from trial to trial must be due to the difference between years, or to the lack of environmental influence on pulse rate. When interpreted in the light of the findings of other workers, year differences alone do not account for enough of the variation. Inconsistency with respect to pulse rate is a common observation and is causing pulse rate to lose favor as a useful measure in physiological studies of this type.

The three main effects for both years in the analysis of variance of the data taken on respiration rate were highly significant. The large mean square for days is accounted for easily by the fluctuations in the daily environment. The significant mean squares for locations and for genetic groups demonstrate the influence of variation in solar radiation and genetic background in causing differences in physiological response.

The two within-locations analyses and the two within-genetics-groups analyses show definite differences attributable to solar radiation and genetic origin with respect to respiration rate. Since respiration rate is accepted widely as a good indicator of heat distress, particularly in climates similar to the one in which this work was conducted, the evidence presented here demonstrates that solar radiation is a major cause of heat distress.

When the results of the analyses of variance of the three physiological responses are combined and interpreted over the 2 yr., body temperature and respiration rate are far superior to pulse rate as indicators of differences due to solar radiation and genetic origin. When pulse rate results are omitted from consideration, the remaining analyses of variance illustrate appreciable variation due to differences between locations within groups and to differences between groups within locations.

The multiple correlation coefficients representing the influences of the en-

vironmental variables on body temperature for the Jersey heifers (Table 4) show that there is a gain in predictive value attained by using sun measurements, and especially by using sun measurements taken in air temperatures above 90° F. The Jersey cattle were observed to show a relatively high degree of heat regulation.

Measurements of the Holstein heifers, like those of the Jersey heifers, produced the largest multiple correlation coefficient in the sun locations in air temperatures above 90° F. However, unlike the Jersey, the next highest value was obtained in the shade location in air temperatures below 90° F. This may be due to an error in sampling, or possibly it is a direct result of the Holstein's anatomy and physiology. The large body size and the large variation in surface area of the black, heat-absorbing hair coat may cause the Holstein cattle to be relatively more variable in normal body temperature within their breed.

Unlike the Holstein and Jersey heifers, body temperatures of the heifers having Brahman ancestry were not affected severely by large ranges of climatic variation. The multiple correlation coefficients (Table 4) indicate small individual, but cumulatively important, effects of environment. From the statistics calculated, the best indications of the effects of environment are obtained when solar radiation is included in the measurements.

Using the multiple correlation coefficient as a guide, the differences in body temperature responses between the European and Brahman cattle were consistently evident. After combining estimates for all genetic groups and atmospheric temperature levels, there remained only a slight difference between sun and shade values. Observed without the crossbred heifers, this difference is magnified for the higher air temperature level, and substantiated the results of a preliminary examination of standard partial regression coefficients. The explanation is that one of the reasons the crossbred animal has superior heat tolerance is its ability to resist the effects of solar radiation. Previous research has attributed this resistance to hair coat characteristics [Bonsma (1), Rhoad (12), Riemerschmid (14), Riemerschmid and Elder (15), and Findlay (3)].

When genetic groups and locations were examined together within a temperature level, multiple correlations were higher in the 90° F.-and-higher range of atmospheric temperatures. Because this range is above the commonly accepted threshold for heat distress, the larger coefficients obtained can be attributed to the rapidly increasing responses to air temperature. (That the effects of solar radiation and vapor pressure on body temperature apparently are suppressed by the high atmospheric temperatures was observed in the examination of standard partial regression coefficients.)

Again, the inconsistency of analyses of the pulse rate data complicates interpretation of the results. The multiple correlation coefficients representing the combined influences of the environmental variables on pulse rate are particularly difficult to interpret. The 1955 results (Table 5) indicate a definite relationship among the variables, which was increased when readings were taken in the sun. The 1956 data showed very large values of relationship in atmospheric temperatures less than 90° F., but when the atmospheric tempera-

ture exceeded 90° F. in 1956, these relationships disappeared. No explanation other than the slight effect of solar radiation and its reduced effectiveness in air temperatures above 90° F. can be offered.

The respiration rate data used in these analyses consistently gave the most uniform statistical results. In Table 6, the accumulation of the individual effects is illustrated. For both years, the data are consistent between the two temperature levels. Uniformly, in both years and in both air temperature levels, the increase in information gained by using the sun location was evident. The smallest differences in the multiple correlation coefficients were among genetic groups. This, however, can be explained when years are studied separately.

In the first year, the three genetic groups were observed to be fairly heat-tolerant. Previous studies have indicated that Jersey heifers, although less adapted than crossbred heifers such as those used here, are able to withstand high air temperatures and high solar radiation relatively well as compared to other European breeds, particularly Holsteins, which can account for the small range in magnitude of the multiple correlation coefficients. When Holstein heifers were used in 1956, the range in magnitude of the coefficients increased, because these heifers were apparently affected more adversely by the local climate. This was especially noticeable at the atmospheric temperatures below 90° F.

Apparently, body temperature is influenced appreciably by air temperature. Because it is nearly constant under nonstress conditions, it is the best indicator of changes in the physiology of an animal. The animal that can maintain a nearly constant and relatively low body temperature in high atmospheric temperatures should be a desirable and heat-tolerant type. As respiration rate initially increases with body temperature, respiratory-muscle activity contributes additional body heat. When exposed to climatic extremes, this relationship is accentuated and becomes heat distress.

Pespiration rate is dependent, however, according to the present results, upon both solar radiation and atmospheric temperature. Therefore, directly or indirectly, just before and after the threshold levels of heat distress have been exceeded, solar radiation is only slightly less important than air temperature.

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A STUDY OF SOLAR RADIATION AS RELATED TO PHYSIOLOGICAL AND PRODUCTION RESPONSES OF LACTATING HOLSTEIN AND JERSEY COWS[1]

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SUMMARY

The influence of summer weather conditions in central Texas upon lactating Holstein and Jersey dairy cattle was studied. Analysis of variance and multiple regression methods were used to study the effects of weather factors upon milk production and body temperature, respiration rate, and pulse rate responses of these animals. Weather factors studied were ambient temperature, solar radiation, and vapor pressure. Breed comparisons and comparisons between animals exposed to direct solar radiation and those shaded from direct solar radiation were made. Parts of this study were further subdivided into high and low ambient temperature groups.

Body temperatures, pulse rates, and respiration rates increased during exposure to direct solar radiation throughout a summer day. Body temperatures and respiration rates were higher and pulse rates were lower for individuals of the Holstein breed than for individuals of the Jersey breed.

Air temperature variations were the predominant cause of variations in respiration rate and body temperatures, while solar radiation was of considerable importance as a direct cause of increased body temperatures and respiration rates of animals exposed to the sun. For shaded animals, increases in solar radiation caused no appreciable direct influence upon these physiological responses. Although slight increases in body temperature and respiration seemed to be associated with increases in vapor pressure, this influence, under the conditions of this test, was slight in comparison with that of the other environmental factors. The influence of the environmental factors upon body temperature and respiration rate was considerable, while the influence upon pulse rate responses was so slight that consistent results could not be obtained. The body temperatures and respiratory activities of Holstein individuals and individuals located in the sun were influenced to a greater degree by the environmental conditions than were the same measurements of Jerseys and shaded animals.

Although exposure to direct solar radiation had considerable effect upon the physiological measurements, it did not cause an appreciable change in milk production. The relationship between the physiological responses and milk production, therefore, may not be as great as is generally assumed, and merits further study.

For the past 20 yr., numerous observations and experiments have been made upon the reactions of cattle to tropical and subtropical environments. The general purpose of these studies has been to determine the reasons for certain animals and breeds being better able to withstand the stressing effects of such environments, with the primary point of interest being the physiological re-

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sponses of the animals to various environments in the laboratory and in the field. A comprehensive review of the literature on these studies has recently been made by McDowell (3), and Johnston (1) has reviewed the literature on the effects of high temperatures on milk production. The relationship between the physiological responses to environmental factors and the effects of environmental factors on production has not been definitely established. However, it has been generally assumed that the physiological responses, body temperature, and respiration rate indicate the degree of efficiency of the thermoregulatory processes of the body and, therefore, the ability of an animal to continue satisfactory economic performance under heat stress. The effects of the environmental factors, air temperature and vapor pressure, have been studied in some detail. Information on the influences of solar radiation, however, is limited. Shrode *et al.* (5) and Williams *et al.* (7) found that the importance of variation in solar radiation in producing physiological responses was comparable to that of variations in air temperature, and was considerably more important than vapor pressure and wind velocity under the field conditions with which they worked.

The study reported here was conducted in an effort to gain further insight into the effects of variation in solar radiation, as well as air temperature and vapor pressure, upon the physiological responses and production of lactating Holstein and Jersey cattle.

EXPERIMENTAL PROCEDURE

The data used in this study were collected at College Station, Texas, in August of 1957. The climatic conditions during the test may be described as consisting of moderately high temperatures (daily maximum temperatures in the upper nineties), with considerable humidity and solar radiation accompanied by consistently mild breezes and occasional cloudiness. The experiment was conducted on lactating cows, six Jerseys and six Holsteins. These cows ranged from 4 to 8 yr. of age, and their stages of lactation ranged from 6 to 10 mo. Most of them had been rebred.

On eight test days, six cows, three from each breed, were haltered and tied so as to be completely exposed to the sunlight, while the remaining six animals were restrained under 9.5- by 12-ft. galvanized metal shades, which were 8 ft. above sod-covered ground. These shades were the ones used in the study reported by Williams *et al.* (7). Preliminary observations indicated that air temperature under these shades at cow level was the same as in open shade. Either one or two days elapsed between test days, and the individual cows were alternated between sun and shade locations on successive test days. Additional restraining ropes were required to keep the cows in the shade locations completely shaded throughout the day. The cows were not allowed access to feed during the test periods, but at mid-day were offered water, which some of them refused.

Beginning at 6 A.M., hourly physiological measurements were made until 5 P.M. Rectal temperature was measured with a standard veterinary thermom-

eter and recorded to the nearest one-tenth of a degree Fahrenheit. Pulse rate measurements were based on a 10-sec. palpation of the samhenous artery, and respiratory counts were made by visually observing flank movements for a period of 20 sec. After 5 P.M. the test cows were fed and milked. Free movement and access to feed and water were then allowed until the next test day. All of the morning and night milk weights were recorded to the nearest one-tenth of a pound for the test cows, as well as for all individuals in the milking herd.

Continuous measurements of solar and sky radiation were made by means of a pyrheliometer; a thermal anemometer was utilized to measure wind velocity. Air temperature and relative humidity were recorded continuously by a hygrothermograph.

Because the anemometer did not function correctly on three of the test days, data on wind velocity were incomplete, and this variable was, therefore, not used in the present study. Experiments during the four previous summers at this same station [Shrode *et al.* (5) and Williams *et al.* (7)] yielded coefficients of correlation between wind velocity and physiological measurements, all of which were either nonsignificant or of low magnitude. Wind velocity, therefore, was probably not of much importance in this experiment, and very little has been lost as a result of not including it as a causative factor in the analyses.

For each of the three environmental variables studied, hourly mean estimates were made for each hour immediately preceding the recording of physiological measurements. The values for relative humidity were converted to vapor pressure estimates by methods made available by List (2).

The milk weight data for each milking of each cow were converted to the ratio of actual production to expected production, and recorded. The expected production was estimated from the mean production of each cow at morning and at evening milkings during a base period of 1 mo. preceding the tests, with the mean production adjusted by breed estimates of per cent decline per day in production, i.e.,

$$\text{Cow's expected production} = \begin{matrix}\text{Cow mean}\\ \text{for base}\\ \text{period}\end{matrix} \times \left[1 - \left(\begin{matrix}\text{Fractional}\\ \text{decline per}\\ \text{day for}\\ \text{the breed}\end{matrix}\right)\left(\begin{matrix}\text{No. of days}\\ \text{after base}\\ \text{period mean}\\ \text{date}\end{matrix}\right)\right]$$

with

$$\text{Fractional decline per day for the breed} = \frac{\begin{matrix}\text{Breed regression of milk production}\\ \text{on day}\end{matrix}}{\begin{matrix}\text{Mean production of base period for}\\ \text{individuals of the breed}\end{matrix}}$$

The coefficient of regression of milk yield on days was calculated for each breed from the recorded production of other cows in the herd, 25 Holsteins and 26 Jerseys, for a period including the base period and the test days. The 51 other cows were in approximately the same stage of lactation as the test cows. These cows were subjected to the regular routine followed in the herd management, which consisted of several hours on pasture in the early morning,

with access to shade. During the remainder of the sunlight hours, the majority of the time was spent under shaded conditions in the lots and in the stanchion barn. The percentage decline per day was estimated to be 0.224 and 0.197% for Holsteins and Jerseys, respectively.

Analysis of variance methods and multiple regression methods, as described by Snedecor (6), were employed to study the influence of causative factors upon variation in the physiological responses, and analysis of variance methods alone were used to study the variation in the ratio of actual milk production to expected milk production. Even though the investigation of the effect on production was based on ratios, the distribution of the ratios was such that no special statistical treatment seemed indicated.

RESULTS AND DISCUSSION

Analyses of variance of the day totals of each of the physiological responses were conducted and are presented in Table 1. The Holsteins were found to

TABLE 1

Analysis of variance of day totals of body temperature, pulse rate, and respiration rate responses

		Mean squares		
Sources of variation	Degrees of freedom	Body temperature	Pulse rate	Respiration rate
Breeds	1	3,534.44**	1,261.00*	130,316.00**
Locations	1	767.83**	1,080.00*	32,967.00**
Days	(7)	105.11**	781.00**	5,444.71*
Odd vs. even days	1	22.34	2,882.00**	6,617.00
Days within day groups	6	118.90**	430.83	5,249.33*
Breeds × locations	1	80.48	42.00	230.00
Breeds × days	7	17.00	75.57	488.43
Locations × days	(7)	3.83	603.86*	7,103.00**
Location × day groups	1		3,927.00**	46,331.00**
Locations × days within day groups	6		50.00	565.00
Error	(71)	20.97	234.20	2,324.17
Breeds × locations × days	7	19.07	23.57	972.71
Remainder	64	21.17	257.23	2,471.98

*, ** Throughout the tables in this paper single asterisks will denote statistical significance between the 0.05 and the 0.01 levels of probability, and double asterisks will denote statistical significance beyond the 0.01 level of probability.

have highly significantly higher body temperature and respiration rate and significantly lower pulse rate than the Jerseys. In further calculations, not presented here, differences in the same direction for the body temperature and respiration rate measurements were found to occur also under the mildest conditions of the day, i.e., the 6 A.M. measurements when the mean air temperature was 73° F., and solar radiation was effectively nil. The differences were considerably greater, however, for the subsequent hourly mean responses as compared to the 6 A.M. measurements. The study of location differences showed that the individuals completely exposed to direct sunlight exhibited highly significantly higher body temperature and respiration rate and significantly higher pulse rate. These findings suggest that the Holsteins exert greater respiratory activity than the Jerseys in an effort to cope with the additional heat

load due to the elevated temperatures and solar radiation encountered during the day. A higher normal heat load for the Holsteins is suggested by the greater measurements encountered in these individuals at 6 A.M. This greater heat load is probably due to the higher level of production and related physiological factors. The solar radiation impinging on the animals located in the sun also seems to be a source of additional heat load for the animal's body. The increased respiration rates, however, do not seem to eliminate the additional thermal influence, and the body temperatures respond upward for the Holsteins and for the animals located in the sun, as compared to the Jerseys and the shaded animals, respectively.

The higher pulse rates occurring in the sun location suggest an influence of the additional solar radiation upon the circulatory system of the animals. The greater pulse rate responses of the Jerseys are not easily explained, although they may have been due to the handling of the animals for the recording of the measurements during the test days, since Jerseys are generally assumed to be more nervous, on the average, than Holsteins.

Since the animals were alternated between locations on successive days, the difference in responses on the odd test days as compared to the even test days is seen as an additional source of variation, not directly attributable to the differences in environmental conditions on the different test days. The mean squares for days within day groups, therefore, are assumed to be more accurate indicators of the differences in responses due to the varying conditions of the test days. From a study of these values, it can be seen that highly significant body temperature responses and significant respiration rate responses were obtained for the variation due to days, whereas the variation of pulse rate measurements was not significant for this source of variation. Although the mean squares for locations $\times$ days were noted to be significant and highly significant for pulse rate and respiration rate responses, respectively, the location $\times$ days within day groups mean squares for these responses were nonsignificant. As a result, none of the interactions in these studies was found to be an appreciable source of variation.

Because differences between breeds and between locations were noted in the analysis of variance studies, and because differences in magnitude of response at different temperature ranges have been noted by other workers, for multiple regression analysis the data were divided into eight groups. These groupings were for high and low ambient temperatures within the two locations for each of the two breeds. The temperature of 88° F. was selected as the point of division for the temperature ranges, since this value divided the data almost equally and is near the critical temperature suggested by other workers.

The coefficients of standard partial regression as presented in Table 2 were calculated, to study the magnitude of the variation in each of the physiological measurements attributable to regression on each of the environmental variables, independent of the influence of variation of the other two environmental variables. Although these coefficients have not been tested for statistical significance, their relative size is useful in evaluating the importance of the relationships.

TABLE 2

Coefficients of standard partial regression of physiological responses on environmental variables and coefficients of multiple correlation among environmental variables and physiological responses for different groupings of the data

Breeds Locations Temperature groups	Holstein Sun 88° F. or above	Holstein Sun Below 88° F.	Holstein Shade 88° F. or above	Holstein Shade Below 88° F.	Jersey Sun 88° F. or above	Jersey Sun Below 88° F.	Jersey Shade 88° F. or above	Jersey Shade Below 88° F.
$b'_{ta \cdot av}$	+.1353	+.3344	−.0716	+.1879	+.2035	+1.2962	+.0232	−.0318
$b'_{ta \cdot sv}$	+.6468	+.5351	+.8110	+.4823	+.6315	− .6176	+.0710	+.4365
$b'_{tv \cdot sa}$	+.0780	+.1251	+.3612	+.3393	+.0269	+ .1924	−.2143	+.0419
$b'_{ps \cdot av}$	+.2634	−.4063	+.0080	+.0429	−.0806	+ .4623	+.3272	−.1899
$b'_{pa \cdot sv}$	−.1628	+.2573	−.0670	+.1811	+.3772	− .2369	+.1895	+.1756
$b'_{pv \cdot sa}$	−.2160	−.1412	+.0634	+.0828	+.3104	+ .0173	−.0552	−.0234
$b'_{rs \cdot av}$	+.4018	+.4126	+.1166	+.3096	+.4486	+1.1123	+.2922	−.2129
$b'_{ra \cdot sv}$	+.5370	+.4700	+.1602	+.4833	+.3535	− .2744	−.2738	+.8258
$b'_{rv \cdot sa}$	+.0828	+.0731	−.2919	+.1390	+.1203	+ .2036	−.2989	+.0401
Degrees of freedom	100	89	100	89	101	89	101	89
$R_{t \cdot sav}$	+.5406**	+.8306**	+.5774**	+.6399**	+.5525**	+ .6271**	+.2659*	+.4050**
$R_{p \cdot sav}$	+.2582*	+.0504	+.1288	+.2113	+.2287	+ .2284	+.3029**	+.0113
$R_{r \cdot sav}$	+.4747**	+.8551**	+.3904**	+.7527**	+.4270**	+ .7863**	+.2257	+.6092**

In this table, b′ denates standard partial regression coefficient; R, multiple correlation coefficient; a, air temperature; s, solar radiation; v, vapor pressure; t, body temperature; p, pulse rate; and r, respiration rate.

A comparison of the values indicates that under most conditions increases in air temperature appeared to be the major cause of rises in body temperature. For the animals exposed to the sun, increases in solar radiation were second in importance, while increased vapor pressure was second in importance in increasing body temperatures of the shaded animals. Solar radiation was included as an independent variable in the multiple regression analysis of data from the shade location. However, it apparently had very little or no effect upon the animals in the shade, although Riemerschmid (4) reported that the amount of radiation absorbed by shaded animals was 30 to 40% of the amount impinging on the animal's body in direct sunlight.

The respiration rate responses closely paralleled the body temperature responses. For animals exposed to the sun, both increases in solar radiation and increases in air temperature were apparent causes of considerable amounts of increase in respiratory responses, whereas vapor pressure had relatively little influence. Shading of the animals reduced the influence of solar radiation considerably, with the result that air temperature was the apparent cause of the larger portion of the variation in respiration rates in the shade. The influence of vapor pressure was not consistent, although, over-all, increases in vapor pressure seemed to cause slight increases in respiration rate.

The coefficients of standard partial regression involving pulse rate as the dependent variable were, in general, non-uniform in sign and of low magnitude. It seems that although the environmental variables might have affected the pulse rate responses, these effects were so slight in comparison with the effects of other factors not considered in this experiment, that very little of the variation in pulse rate could be accounted for by the variation in the environmental

variables studied. For these reasons, pulse rate responses can not be considered effective indicators of the influences of climatic conditions upon the physiological processes of dairy cattle.

The coefficients of multiple correlation, also presented in Table 2, were studied as indicators of the over-all influences of the environmental variables upon the physiological responses. The magnitude of these values for body temperature and respiration rate attests to the considerable influence of the environmental variables upon these responses. On the other hand, these values for the pulse rate measurements are relatively small, with only one value being statistically significant and one statistically highly significant. The latter value seems to be due to an unexplained relationship between pulse rate and solar radiation for shaded Jerseys at high ambient temperatures. This value seems not to be consistent with the other values observed, and no plausible explanation is evident.

Since the analysis of variance studies showed that the body temperature and respiration responses were higher for the Holstein animals, and for the animals located in the sun, it is not surprising that the coefficients of multiple correlation are higher for the groupings of the data involving these groups, as compared to those for the Jersey and shade-location groups. The larger coefficients of multiple correlation for the ambient temperatures below 88° F., as compared to those for the higher temperatures, seem to conform to the pattern of earlier studies, in which it was evident that variables other than air temperature have reduced effectiveness as air temperature increases. Air temperature, the most influential variable, as it increases, causes the cattle to approach more and more closely the limit of their ability to respond, leaving less and less freedom to respond to variables other than air temperature.

The analysis of variance of the milk weight data, as presented in Table 3, indicates that the decline in production when studied on a fraction--of-normal-production basis, was considerably greater for the Jerseys than for the Holsteins. The differences in the ratios of actual milk weight to expected milk weight among the test days were found to be significant. The difference due to the location of the animal was found to be nonsignificant; that is, there was no significant difference in the decline of production between the shade location and the sun location. This finding is contrary to expectation and should be confirmed by additional work before any involved plausible explanation is proposed. At first glance, the greater decline in production for the Jerseys seems surprising, although further consideration suggests that this greater decline may have been due to greater reactions to the handling of the animals during the test, rather than to the recorded environmental variables alone. The nonsignificant locations mean square suggests that although exposure to solar radiation as compared to shading exerted considerable influence upon the physiological measurements, the influence upon the milk production of the animals was not appreciable. The highly significant mean square for milkings following exposure shows that considerable variation existed in the decline for the four milkings following the test days, and examination of the mean ratios

TABLE 3
Analysis of variance of the ratio of actual milk production to expected milk production

Source of variation	Degrees of freedom	Mean square
Breeds	1	3,621.74**
Locations	1	144.92
Days	(7)	414.63*
Odd vs. even days	1	446.99
Days within day groups	6	409.23*
Milkings following exposure	3	3,785.05**
Breeds × locations	1	38.68
Breeds × days	7	230.09
Breeds × milkings	3	137.05
Locations × days	(7)	938.33**
Locations × day groups	1	5,765.22**
Locations × days within day groups	6	133.84
Locations × milkings	3	99.33
Days × milkings	21	270.98
Breeds × locations × days	(7)	491.93**
Breeds × locations × day groups	1	2,887.00**
Breeds × locations × days within day groups	6	92.75
Breeds × locations × milkings	3	57.54
Breeds × days × milkings	21	64.91
Locations × days × milkings	21	66.19
Breeds × locations × days × milkings	21	89.85
Remainder	256	168.54

shows that the decline in production for the morning milking on the day following the test day was considerably the greatest. When the variation due to the grouping of the animals was removed, none of the interactions was found to be an appreciable source of variation.

These findings suggest the necessity for a re-evaluation of the relationship between the physiological responses and the production of animals exposed to extreme environmental conditions. It seems, from this study, that the physiological responses are not effective indicators of the influences of the environmental variables, particularly solar radiation, upon the production of a dairy animal. Naturally, the influence upon an animal's production is the primary point of interest of the dairyman.

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VARIATIONS IN CERTAIN BLOOD COMPONENTS OF HOLSTEIN AND JERSEY COWS AND THEIR RELATIONSHIP TO DAILY RANGE IN RECTAL TEMPERATURE AND TO MILK AND BUTTERFAT PRODUCTION[1]

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SUMMARY

Winter and summer blood samples were taken from 96 Jersey and 78 Holstein cows. Laboratory determinations of the levels of reduced glutathione and hemoglobin and of red-cell volume were made. The data were analyzed statistically to determine all correlations of possible interest and meaning, to determine the relative importance of various assumed causes of variation in the blood components and in daily range in rectal temperature, another variable of interest observed on cows in milk during the summer.

All of the blood components were significantly repeatable. Age variation seemed to have no influence on variation in the blood components or daily temperature range. Breed and season differences were significant, but significant sire differences were not observed, except in the case of glutathione in the Holstein breed. Stage of lactation and/or stage of gestation appears to have an influence on or be related to hemoglobin and daily temperature range, stage of lactation being the more important in Jerseys and stage of gestation the more important in Holsteins.

Correlations of the blood components with most probable performing ability for milk production, fat production, and fat test were so small that the blood components were concluded not to be useful criteria of selection for these economic traits.

With reference to farm animals, most traits of economic importance, such as rate of gain, or milk and butterfat production, are quantitative in nature and are polygenic in their inheritance. In addition, many of these traits are low in heritability, which makes it difficult to identify animals which will have the best inheritance for these traits. It becomes apparent that some method, in addition to direct phenotypic selection, is necessary to more accurately evaluate the true breeding worth or transmitting ability of those individuals being considered for selection as breeding stock.

Progeny testing and consideration of phenotypes for correlated traits are often used under these circumstances. Of the two, the latter may be more desirable because of the shorter time lapse before selection is made, provided the correlated trait can be measured easily, accurately, and at an early stage in the life cycle, and provided the correlation is of sufficient magnitude.

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Because of its widespread occurrence in the tissues and fluids of the body, the tripeptide glutathione has been postulated as an intermediate step in the synthesis of proteins from amino acids. Recently, Kunkel *et al.* (11) showed that the level of reduced glutathione in the blood of young beef animals is a characteristic of each animal. Evidence that the glutathione concentration within the erythrocytes of mature chickens is under genetic control was presented by Stutts *et al.* (24). Negative correlations of glutathione and glutathione-hemoglobin ratio with egg production were found by Stutts and coworkers (23) in inbred lines of chickens. The relationship of glutathione to growth has been shown in several species: in the pig by Wilkerson and Gortner (26), in the rabbit by Gregory and Goss (6, 7) and Lerner *et al.* (14), in the chicken by Gregory and associates (8, 9), and in cattle by Patrushev (19, 20).

Ling and Chow (15) reported that all of the sulfhydryl compounds of the blood could be accounted for by the red blood cell glutathione level. It was suggested by Kunkel *et al.* (11) that the differences in reduced glutathione level in the blood of young beef animals can be accounted for in part by the differences in numbers of red cells, as measured by hemoglobin concentration. These associations suggest a study of hemoglobin and its apparent relationship with glutathione. Several workers, McCay (18), Brook and Hughes (2), and Rusoff *et al.* (21) have found no differences in hemoglobin level due to breed differences. On the other hand, Buyers *et al.* (3) reported a significantly higher hemoglobin level in the Jersey breed as compared to the Holstein breed. Anderson *et al.* (1) reported a breed difference, but found the Holsteins rather than Jerseys to have the higher value. Kushner (13) reported a breed difference which he attributed to differences in size.

Cook and Harmon (4) and Tanaka and Rosenberg (25) reported a negative relationship between hemoglobin and egg production. Other workers, Dukes, Schwarte, and Brandt (5), working with laying hens, and Kushner (12) and McCay (18) with cattle, found no relationship between hemoglobin and production.

The objective of the present investigation was to determine the inter-relationships and relative influences of differences in sire, breed, size, age, stage of lactation, season of year, daily temperature range on the variation in blood levels of reduced glutathione, red-cell volume, and hemoglobin. Finally, heritability estimates were calculated for blood level of glutathione, hemoglobin content, and red-cell volume.

MATERIALS AND METHODS

The experimental cattle used in this investigation were cows of the Holstein and Jersey breeds, all of which had at least started their first lactation period before the study was concluded.

Present in the herd, both at the beginning and at the end of the investigation, were 174 cows, consisting of 78 Holsteins and 96 Jerseys. All of the cows were subjected to the same management practices, and the collection of the desired data did not alter or interfere in any way with the management of the herd.

An effort was made each day to secure the blood samples immediately after the morning milking, so that the cows' milk production would be disturbed as little as possible. Since the length of time necessary for each determination prevented the taking of all the blood samples on the same day, for the first series they were taken over the period extending from February 28, 1955, through March 30, 1955. The initial samples of the second series were taken on July 7, 1955, and the final samples of that series were collected on the following August 4. The samples were collected by venepuncture into tubes containing potassium oxalate as the anticoagulant, and after thorough mixing of the blood with the oxalate, the tubes were placed immediately into an ice bath and held at approximately 0° C. until the laboratory analyses were begun.

The level of reduced glutathione was determined from whole blood by the method of Grunert and Phillips (10). Duplicate analyses were made on each sample on the same day it was collected.

Hemoglobin was determined colorimetrically as oxyhemoglobin, and the red-cell volume was determined after centrifugation of the blood for 15 min. at a force 1,200 times gravity.

In addition to the data on blood constituents, age, stage of lactation, and stage of gestation were available. The heart girth of each cow was measured, as it was believed that this measurement presents a better estimate of the relative size of each cow than would a weight taken at any particular time. Also, the most probable performing ability (m.p.p.a.), Lush (16), was calculated for each of the three variables: milk production, butterfat production, and butterfat test (hereafter referred to as milk, fat, and test, respectively). Repeatability values used in these calculations were 0.5 for milk, 0.4 for fat, and 0.7 for test, and conform to the results of Shrode *et al.* (22), who calculated estimates from earlier data from the herd on which the present study was conducted. Additional production information was obtained both by calculating the production of milk and fat and the average test for the 15 days preceding and the 15 days following the date of bleeding for each cow at each bleeding date. These values will be referred to hereafter as current milk, current fat, and current test, respectively.

Immediately following the last series of blood collections, average daily temperature ranges for each cow in the milking herd were determined. Rectal temperature was recorded just prior to the morning and the afternoon milkings. The difference between the two readings was considered as the daily temperature range. Temperatures were taken on alternate days, and only the cows observed for five or more days were considered.

Averages, standard errors, and coefficients of variation for all but the production variables are presented in Table 1, to give the reader a general impression of the situation in the herd with respect to these less commonly discussed variables.

The relationships that existed among the different variables described above were expressed as product-moment correlation coefficients and were determined by the methods described in various standard textbooks. Estimates of repeat-

TABLE 1

The mean, standard error, and coefficient of variation of each blood component, stage of gestation, stage of lactation, and daily temperature range

Variable	Breed	Season	Mean	Standard error	Coefficient of variation
Glutathione (*mg/100 ml.*)	Holstein	1	31.81	0.68	18.74
		2	26.44	0.61	20.46
	Jersey	1	34.09	0.56	16.10
		2	28.97	0.51	17.26
Hemoglobin (*g/100 ml.*)	Holstein	1	11.08	0.11	8.48
		2	10.10	0.10	8.71
	Jersey	1	10.96	0.12	10.77
		2	10.43	0.11	10.55
Glutathione-hemoglobin ratio	Holstein	1	2.86	0.05	16.43
		2	2.63	0.06	19.77
	Jersey	1	3.11	0.04	11.58
		2	2.78	0.04	15.11
Red-cell volume (*%*)	Holstein	2	0.34	0.003	8.82
	Jersey	2	0.34	0.004	11.76
Stage of gestation (*days*)	Holstein	1	95.2	8.14	57.94
		2	162.1	11.21	47.94
	Jersey	1	86.1	7.16	68.48
		2	153.9	8.28	50.23
Stage of lactation (*days*)	Holstein	1	187.8	12.28	63.91
		2	231.7	10.22	41.86
	Jersey	1	181.6	16.69	65.48
		2	242.8	11.15	35.86
Daily temperature range (*° F.*)	Holstein	2	1.14	0.06	33.33
	Jersey	2	0.80	0.04	40.00

ability of successive determinations were based also on product-moment correlations. Estimates of heritability were based on half-sib correlations by the method described by Lush (17).

RESULTS AND DISCUSSION

Correlation studies. The precautionary measure of making duplicate determinations for reduced glutathione was taken. Since it is necessary to use the average of the two determinations on each animal in correlating glutathione level with other variables, it is desirable to demonstrate the small amount of error of measurement involved in the laboratory determinations; that is, the variation between duplicate determinations on the same sample. This demonstration was accomplished by calculating the standard error. The relatively small size of the standard error of 0.58 for differences between determinations on the same individual, as compared to the standard error of 7.71 for differences between individuals of the same breed, indicates very little difference between the two values obtained for each animal. Further, the greatest differences between two determinations were 3.21 and 3.58 mg. % for the Jersey and Holstein breeds, respectively.

TABLE 2
Repeatability of successive measurements of various blood components

Component	Holstein	Jersey	Combined
Glutathione	0.58**	0.49**	0.56**
Hemoglobin	0.22**	0.51**	0.40**
Glutathione-hemoglobin ratio	0.71**	0.46**	0.66**

** Throughout this paper double asterisks, as above, will denote statistical significance beyond the 0.01 level of probability; single asterisks will denote significance between the 0.05 and the 0.01 level of probability.

Table 2 shows the estimates of repeatability of determinations of the various blood constituents, based on a combination of the data from the two breeds used in this study, and also for each breed separately. These estimates are product-moment correlations between the winter and summer values for each animal. In the Holstein breed, it is worthy of note that the repeatability for hemoglobin is relatively low, as compared to the very high repeatability for the glutathione-hemoglobin ratio.

The correlations found among the various blood constituents are presented in Table 3. The low correlation obtained for Holsteins during the summer

TABLE 3
Correlations among the blood components

Component	Breed	Season[a]	Glutathione	Hemaglobin	Glutathione-hemoglobin ratio
Hemoglobin	Holstein	1	0.527**	.....	.....
	Jersey	1	0.691**	.....	.....
	Holstein	2	0.136	.....	.....
	Jersey	2	0.487**	.....	.....
Red-cell volume	Holstein	2	0.296**	0.821**	−0.086
	Jersey	2	0.516**	0.840**	−0.006

[a] Hereafter in this paper, Season 1 refers to the winter bleeding series and Season 2 refers to the summer bleeding series.

season appears to be a reliable estimate; that is, it is not affected greatly by sampling error. Supporting this conclusion is the low correlation between red-cell volume and glutathione found in using data collected from the same animals at the same time. This result seems reasonable, when it is remembered that hemoglobin is a measure of red-cell volume, as indicated by the high correlation between these two variables for both breeds.

The low correlations between red-cell volume and glutathione-hemoglobin ratio are to be expected, since the effects of variation in red-cell volume have been removed from the ratio by virtue of the division of the glutathione value by the hemoglobin value.

Included in Table 4 are the correlations of the various blood constituents and daily temperature range with certain variables that, according to some reports in the literature, were thought to be related to the blood values. The consistency of the results obtained from the data of the present study for the winter season is of interest with respect to both glutathione and hemoglobin.

TABLE 4

Correlation of the various blood components and daily temperature range with certain physiological factors

Component	Breed	Season	Stage of lactation	Stage of gestation	Age in months	Heart girth in centimeters	Daily temperature range
Glutathione	Holstein	1	0.193	0.138	0.133	0.031	.....
	Jersey	1	0.241*	0.386**	−0.076	0.161	.....
	Holstein	2	0.058	−0.102	0.239*	0.031	0.162
	Jersey	2	−0.100	−0.044	−0.028	0.204*	−0.103
Hemoglobin	Holstein	1	0.473**	0.426**	−0.134	0.073	.....
	Jersey	1	0.366**	0.478**	0.011	0.142	.....
	Holstein	2	0.300**	−0.016	0.017	0.205	−0.215
	Jersey	2	0.079	0.237*	0.003	0.136	−0.070
Glutathione-hemoglobin ratio	Holstein	1	−0.023	−0.066	0.233*	−0.003	.....
	Jersey	1	0.020	0.129	−0.116	0.093	.....
	Holstein	2	−0.039	−0.100	0.240*	0.016	0.266
	Jersey	2	−0.165	−0.205	−0.047	0.145	−0.055
Red-cell volume	Holstein	2	0.277*	0.065	−0.018	0.174	−0.108
	Jersey	2	−0.028	0.302**	−0.068	0.199*	−0.031
Daily temperature range	Holstein	2	−0.361*	0.426	0.229	0.071	.....
	Jersey	2	−0.461**	0.198	0.099	0.017	.....

It should be pointed out, however, that the correlations that apparently exist involving stage of gestation and stage of lactation with glutathione and hemoglobin are due to the differences associated with red-cell volume, and not to the concentration of glutathione per cell. This conclusion is supported by the fact that the correlations of the glutathione-hemoglobin ratio with stage of lactation and stage of gestation do not differ significantly from zero.

The results obtained during the summer season were not consistent with those obtained during the winter season. This inconsistency may be due in part to a reduction in variation associated with stage of gestation and stage of lactation during the summer. This reduction in variation could account also for the smaller correlations observed with respect to these variables. Further, it was noted that there was a difference in the number of cows contained in each gestation-lactation group for the two seasons. It should be remembered also that stage of gestation and stage of lactation are confounded, making it impossible to evaluate accurately the individual effects of either of the two variables.

A possible cause of the differences which exist between the correlations for the two seasons may be related in some manner to the difference in heat tolerance that is commonly believed to exist between the Jersey and Holstein breeds. In the present study, however, both the Jersey and Holstein breeds showed a slight decrease in hemoglobin during the summer season.

There was little evidence that a relationship exists between age and any of the blood constituents. Since each of the animals involved in the present study was at least 30 mo. old, no change due to increasing age was anticipated.

The most consistent correlations were found between body size, as indicated by heart girth and the blood components. Although only two correlations were

significant, all were positive except one, which was extremely small. These findings are in keeping with numerous reports in the literature.

That the blood components studied have little value for predicting long-range production is evidenced by the small correlations involving m.p.p.a. for milk, fat, and test. It is of interest, however, that the correlations of current milk and of current fat with hemoglobin are all negative and, with the exception of that for Jerseys in the summer season, all are significant.

In the Jersey breed, daily temperature range is significantly correlated with current milk and current fat ($P < 0.01$), and at the same time there is a small nonsignificant negative correlation of hemoglobin with current milk and with current fat (Table 5). In the Holstein breed, on the other hand, the correla-

TABLE 5

Correlation of the various blood components and daily temperature range with factors of economic importance

Component	Breed	Bleeding	Milk (m.p.p.a.)	Fat (m.p.p.a.)	Test (m.p.p.a.)	Current milk	Current fat	Current test
Glutathione	Holstein	1	0.133	0.106	−0.017	−0.021	−0.002	0.166
	Jersey	1	−0.101	−0.128	−0.224*	−0.237*	−0.262*	−0.035
	Holstein	2	0.092	−0.004	−0.120	0.058	0.068	−0.036
	Jersey	2	−0.136	−0.093	−0.130	−0.102	−0.089	0.120
Hemoglobin	Holstein	1	0.025	0.076	0.186	−0.439**	−0.342**	0.523**
	Jersey	1	−0.150	−0.216*	−0.117	−0.404**	−0.436**	0.014
	Holstein	2	−0.062	−0.019	0.106	−0.303**	−0.226**	0.255*
	Jersey	2	−0.088	−0.114	−0.087	−0.130	−0.144	−0.082
Glutathione-hemoglobin ratio	Holstein	1	0.146	0.088	−0.117	0.207	0.180	−0.076
	Jersey	1	0.008	0.024	−0.192	0.021	0.022	−0.048
	Holstein	2	0.109	0.000	−0.150	0.159	0.141	−0.125
	Jersey	2	−0.095	−0.018	−0.031	−0.037	−0.006	0.197
Red-cell volume	Holstein	2	−0.012	0.005	0.149	−0.277*	−0.184	0.299
	Jersey	2	−0.032	−0.069	−0.112	−0.087	−0.124	−0.077
Daily temperature range	Holstein	2	−0.082	−0.022	0.076	0.278	0.117	0.033
	Jersey	2	0.112	0.177	−0.104	0.526**	0.371**	−0.246

tions of hemoglobin with current milk and with current fat are significant ($P < 0.01$ and $P < 0.05$, respectively), while small nonsignificant correlations of daily temperature range with current milk and with current fat are indicated. It would, therefore, be of academic interest to observe multiple correlations among these variables with current milk and current fat as dependent variables and with hemoglobin and daily temperature range as independent variables. In Jerseys, the multiple correlation involving hemoglobin, daily temperature range, and current milk was 0.535, which is highly significant. A correlation of 0.390, also highly significant, was obtained when current fat was considered as the dependent variable. In the Holsteins, the corresponding values were 0.373 ($P < 0.05$) and 0.236; the latter figure is not significant.

Analysis of variance studies. The analysis of variance for each blood component is shown in Table 6. A breed difference is indicated for glutathione and for the glutathione-hemoglobin ratio. The lack of a breed difference for hemo-

TABLE 6
Analyses of variance of the blood components

Source of variation	Degrees of freedom	Mean square		
		Glutathione	Hemoglobin	Glutathione-hemoglobin ratio
Total	347			
Between breeds	1	498.66**	0.93	3.41**
Within breeds	346	36.44	0.94	0.23
Between seasons within breeds	2	941.52**	22.60	3.64**
Between groups within seasons	8	94.45**	6.54**	0.10
Individuals	336	29.67	0.68	0.21

globin leads to the conclusion that the difference in glutathione content observed between the two breeds is due to the difference in concentration of glutathione per red cell. These findings agree with the results of other studies previously cited.

Of further interest is the seasonal difference found for glutathione and the glutathione-hemoglobin ratio. The implication mentioned above, viz., that the observed differences are due to the concentration of glutathione per red cell, is indicated here also. However, consideration of the means of hemoglobin for both seasons (Table 5) will show that in both breeds the hemoglobin level is lower during the summer season than during the winter. Despite the fact that these differences were not large enough to be significant, some of the seasonal differences in glutathione can be accounted for by this difference in red-cell volume. The reports found in the literature on the effects of season of the year on glutathione and hemoglobin are not consistent.

A study of the combined effects of gestation and lactation on the blood components was made by means of an analysis of the differences among the three groups (open, milking; bred, milking; bred, dry). This analysis (Table 5) shows significant differences between groups for glutathione and hemoglobin. It can then be concluded that the differences in glutathione observed for the different gestation-lactation groups are due to the differences in red-cell volume associated with each combination of gestation and lactation status. This finding is confirmed by the significant correlations of hemoglobin level with stage of gestation and with stage of lactation (Table 4).

There is no indication from these data that there is a difference in red-cell volume between the Jersey and Holstein breeds (Table 7). A difference between groups, on the other hand, suggests an effect of gestation and/or lactation.

TABLE 7
Analysis of variance of red-cell volume

Source of variation	Degrees of freedom	Mean square
Total	173	
Between breeds	1	4.23
Within breeds	172	11.48
Groups	4	45.69**
Individuals	168	10.67

These results support the findings with respect to hemoglobin presented above.

Estimates of heritability. Since the method of heritability estimation involving paternal half-sib correlations was to be employed, it was desirable to insure that no influences other than sire effects were contributing appreciably to the differences between groups of paternal half-sibs. If any factor is believed to bias estimates of sire influences, adjustments or special analyses to remove the bias must be used.

Age is an important factor to be considered in this connection, because sire groups are automatically so greatly different in age by virtue of the sires being extremely noncontemporary. Evidence presented earlier (Table 4) indicates that age has little effect on the level of the blood components or on daily temperature range. Hence, age can be safely eliminated from consideration in all cases except in the estimation of heritability for glutathione-hemoglobin ratio in Holsteins.

There was, on the other hand, sufficient evidence to warrant consideration of the effects of stage of gestation and stage of lactation when estimating heritability, with one exception, viz., the estimation of heritability of differences in glutathione-hemoglobin ratio in Holsteins. The method of analysis employed to obtain estimates of the sire and error variance components used in calculating the intra-class correlation between half-sibs is shown in Table 8.

TABLE 8

Analysis of variance

Source of variation	Degrees of freedom	Mean square
Total	N-1	
Between seasons	b-1	
Within seasons	N-b	
Between groups[a] of the same season	g-b	$I + K_s S + K_g G$
Between sires of the same group	s-g	$I + K_s S$
Between daughters of the same sire	N-s	I

I = Variance due to differences between daughters of the same sire.
S = Variance due to differences between sires of the same group.
G = Variance due to differences between groups of the same season.
N = Total number of observations.
b = Number of seasons.
g = Number of groups.
s = Number of sires.

[a] This grouping is based on a combination of gestation and lactation status or an age classification.

Of primary concern were the presence in the Jersey breed of a large number of sire groups which contained only one or two progeny each. It was realized that under these circumstances these individuals would contribute heavily to the variance between sires but little, if any, to the error variance. It was, therefore, decided to remove all sire groups composed of only one or two individuals. When this was done, nine sire groups were removed with the loss of only 12 individuals. It is believed that this procedure vastly improved the accuracy of the heritability estimates in spite of conventional statistical objections to elimination of data. Estimates based on all the data were obviously

biased upward, since some were impossibly large, indicating that the nine sires eliminated were in some way contributing to a positive bias and could not logically be considered to belong to a random group of sires.

Since previous analyses had shown significant breed differences for all variables except hemoglobin and red-cell volume, heritability estimates were calculated for each breed separately. These are presented in Table 9.

TABLE 9

Estimates of heritability

Breed	Glutathione	Hemoglobin	Glutathione-hemoglobin ratio	Red-cell volume
Holstein	0.68	0.00	0.22[a]	0.00
Jersey	0.24	0.10	0.00	0.28

[a] Age differences were removed in calculating this value rather than stage of gestation and stage of lactation.

Only one of the estimates shown was based on a significant variance component for sire differences, viz., that for glutathione in the Holstein data, and this must be kept in mind when these estimates are compared with those from other studies. Since heritability can not be negative, zero appears in the cases where the sire mean squares were smaller than the corresponding error mean squares. It will be noted that the estimate of heritability for glutathione in Holsteins (0.68) exceeds the corresponding estimate of repeatability (0.58), shown in Table 2. No explanation for this, other than that usually proposed in such cases, can be offered here.

The estimate of heritability of 0.68 for glutathione obtained for Holsteins was based on a significant sire difference ($P < 0.05$). This estimate may be compared to a value of 0.86 for glutathione in young Hereford calves, obtained by Kunkel *et al.* (11), and to 0.26, obtained by Stutts (24), in chickens.

Because of obvious known influences of sampling variation and other factors on the heritability estimates obtained here, no definite conclusions can be drawn from these data with respect to the heritability of the traits studied. Perhaps the failure to demonstrate sire differences, if they exist, was due to the small numbers per sire group. The scarcity of numbers necessitated the use of a coarse classification and thus prevented the effective removal of the influences of gestation and lactation from the error variance. Further, even though age effects proved to be nonsignificant, there were some small random differences between age groups that undoubtedly contributed slightly to the error variance. These two possibilities, plus the possibility of large environmental variance among the relatively few members of each sire group, could have accounted for the nonsignificant sire components encountered, and also for the four cases in which the sire mean square was smaller than the corresponding error mean square.

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FORAGE INTAKE AND EFFICIENCY OF FEED UTILIZATION IN DAIRY CATTLE[1]

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SUMMARY

The data from 12 forage experiments, involving 175 Holstein-Friesian cows, were analyzed to study the variation among cows in their forage appetite and the efficiency of feed utilization. Highly significant differences were found among cows in their forage dry matter intake, even after adjustment had been made for milk production, body weight, and daily changes in weight. On a within forage treatment period and year basis the repeatability of the weekly average of forage dry matter consumption was 0.70. Cows tended to maintain their rank in forage consumption when fed several different kinds of forage. The difference among cows in their efficiency of feed utilization was also highly significant. The average efficiency for 20 wk., starting 60 days after calving, was a requirement of 56.7 lb. of TDN for each 100 lb. of FCM produced. Consistent and highly significant differences in efficiency were found in favor of grass silage as compared with hay as a source of forage. Some possible explanations of this are discussed.

In recent years increasing emphasis has been placed on the use of forages for feeding dairy cattle. Dairymen recognize considerable difference in the amount of hay and silage consumed by individual cows. Until recently, little attention has been given to the factors that determine forage appetite, and whether definite progress can be achieved by selecting for this characteristic.

Many of the factors that may influence the total forage intake of cows have been outlined by Mather (17). In studying the variation among cows, adjustment can be made for several factors, such as milk production, body weight, and changes in weight. Also, in well controlled experiments, all of the feed and management factors (type and quality of forage, frequency of feeding, and amount of grain) can be accounted for. If, after making all of these corrections, there is still a variation in forage intake, it is mainly due to physiological differences. Because of such differences, some cows are satisfied with the minimum, whereas others continue to eat until they are filled to capacity. It is in this area that inherited differences may exist. Work is now in progress at Cornell's McDonald Farms to determine the heritability of forage intake.

A second area of research, and one of increasing interest, concerns the efficiency of feed utilization by dairy cows. Although differences in efficiency have been reported by many workers (1, 2, 3, 6, 7, 9, 12, 16), only limited

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practical application has been made of the differences that exist. This failure is mainly due to the lack of an easy, on-the-farm method of measuring efficiency and to the fairly high correlation that exists between efficiency and milk production.

Armsby and Moulton (1) in 1925 defined efficiency on two bases: net efficiency, when the maintenance costs are excluded, and gross efficiency, when maintenance is included. Using the same definitions, Brody (3) did considerable work to determine the efficiency for fairly large numbers of cows.

In view of the increasing necessity to breed cows with high forage appetite and high efficiency of feed utilization, work was undertaken to study these factors. The first step was to analyze the forage experiments conducted at Cornell since 1950, the results of which are reported here. The second step is the current program at McDonald Farms to study forage intake, feed efficiency, and the heritability of these factors, the results of which will be reported later.

EXPERIMENTAL PROCEDURE

The original purpose of the 12 forage experiments that were conducted at Cornell between 1950 and 1958 was to evaluate the harvesting method and the stage of maturity at cutting, as related to the feeding value. The results reported by Slack *et al.* (19, 20), Trimberger *et al.* (21), and Wittwer *et al.* (22, 23) were analyzed for this research. Ten of these experiments were of the change-over design described by Cochran *et al.* (5); two of them were continuous-type trials. The forages consisted either of hay or of grass silage, fed so that the weigh-backs were limited from 2 to 6 lb. of hay and 6 to 15 lb. of grass silage.

The grain-feeding levels used in the change-over experiments were established according to the Morrison feeding standards during the week preceding the experimental period. After this, at the beginning of each week the daily amount of grain fed to each cow was decreased by 0.3 lb. In the original allotment of the cows to the forage treatments, they were balanced for milk production; consequently, under this method, the total amount of grain fed on each of the forage treatments was the same. The grain allowance for each cow in the continuous-type experiments was determined by her production of 4% FCM during the preceding week. Therefore, since each cow was fed strictly according to production, there was variation in the total amount fed to the cows on the various forages. For all of the experiments the grain-feeding rate averaged 1 lb. per 4.1 lb. of 4% FCM.

The experiments comprised four 5-wk. periods. The cows were weighed on three consecutive days at the beginning of the experiment and on the last three days of each of the experimental periods. The digestibility of each forage was determined by the conventional method, with steers. Proximate analysis determinations were made on composite samples of the concentrate and hay. The silage was sampled either weekly or every second week for moisture determination by toluene distillation. The data were analyzed with an IBM 650 electronic computer.

RESULTS AND DISCUSSION

A summary of all of the experimental results, including feed consumption, milk production, body weight, and weight changes is given in Table 1. It can be seen that level of production and consumption was fairly high and that the range among cows was considerable. The table shows also that the cows had an average weight of 1,333 lb. and that the actual amount of hay and silage

TABLE 1

Summary of 12 experiments with a total of 175 cows for 20 wk., starting 60 days after calving[a]

	Forage/day[b]		Daily forage DM intake	Daily grain intake	Daily FCM	Daily change in weight	Average weight
	Hay	Silage					
	(lb.)						
Average	31.6	103.6	26.7	9.6	39.6	+0.24	1333
Range	14.9 to 41.6	82.6 to 130.4	12.9 to 35.8	2.8 to 16.8	18.2 to 65.3	+1.25 to −1.07	1015 to 1605

[a] Average age at beginning of experiments was 4.6 yr.
[b] Only one experimental forage and limited grain were fed daily.

consumed was 31.6 and 103.6 lb., respectively. The average daily dry-matter intake from forage was 26.7 lb. plus a daily grain intake of 9.6 lb. The daily 4% FCM production was 39.6 lb. and the average daily gain in weight, 0.24 lb.

One of the important factors that determines the palatability of hay is the date of cutting. In these experiments the hay was cut at various stages of maturity; Table 2 shows the effect of date of cutting on consumption and digestibility.

The range in cutting dates from June 3 to July 9 showed a progressive change in digestibility from 67.2 to 51.5%, and for consumption a change from 2.72 to 1.95 lb. of hay daily, for each 100 lb. of body weight. The result was that early cutting gave a 39% advantage in hay consumption and an advantage of 82% for daily digestible dry matter intake when early-cut was compared to late-cut forage.

The variation among cows in forage dry-matter intake was analyzed by a covariance analysis, to determine the cow variance and the error variance,

TABLE 2

Effect of date of cutting on consumption and digestibility of hay

Date of cutting	No. of cow-weeks	Daily hay intake		Digestibility %	Daily DDM[a] intake (% of lowest)
		lb. DM/100 lb. body weight	as % of lowest		
June 3–4	160	2.72	139	67.2	182
June 9–10	115	2.64	135	63.1	166
June 11–12	120	2.36	121	65.7	154
June 14–15	470	2.45	126	62.6	153
June 15–18	120	2.28	117	58.5	133
July 1	150	2.30	118	52.7	121
July 5	120	2.13	109	52.2	111
July 7–8	115	2.05	105	52.2	107
July 9	120	1.95	100	51.5	100

[a] DDM–Digestible dry matter from hay.

when adjustment was made for milk production in one case and body weight in another. These analyses showed that there were highly significant ($P < 0.01$) differences among cows in their weekly intake of forage dry matter. The repeatability of weekly consumption was determined as follows: $r = \frac{\sigma 2_b}{\sigma 2_b + \sigma 2_e}$ where $\sigma 2_b$ is the cow variance and $\sigma 2_e$ is the error variance. This is actually the correlation between random observations of the within cow consumption of forage dry matter. The repeatabilities were 0.72 and 0.70 when body weight and milk production were the respective covariates.

Further, a multiple regression was made to simultaneously adjust for all of the measurable variables, which were as follows:

Y = dependent variable = lb. of forage dry matter intake daily.
X_1 = independent variable = lb. of 4% FCM produced daily.
X_2 = independent variable = lb. of body weight.
X_3 = independent variable = lb. of daily weight change.

The regression equation was determined to be:

$$Y = 5.92 + 0.097X_1 + 0.012X_2 + 0.0946X_3.$$

This prediction indicates the pounds of forage dry matter that would be consumed on the average by cows of any value of X_1, X_2, and X_3. The coefficients of each of the variables indicate the change, in terms of pounds of forage dry matter, that will accompany a change of one unit in the variable. For example, a 1,400-lb. cow will consume an average of 1.2 lb. more forage dry matter than will a cow weighing 1,300.

For these data, the multiple correlation coefficient was 0.50. Therefore, R^2 showed that only 25% of the difference among cows in forage consumption was associated with the three independent variables. Highly significant ($P < 0.01$) differences still remained among cows.

A study was made to determine whether cows that are good consumers of one forage respond the same to all forages. Spearman's rank correlations were determined for 60 different pairs of treatments. The average of these values was 0.63. The size of the correlation did not depend on similarity of forages. On an average, three out of four cows in the top half for consumption of one forage were also in the top half when an entirely different forage was fed.

Feed efficiency in this study was defined as the pounds of TDN required for maintenance and the production of 100 lb. of 4% FCM. Adjustment was made for daily changes in weight, but maintenance was included as a requirement, because this is a fixed cost to the animal. Highly significant ($P < 0.01$) differences were found among cows in their efficiency. The average efficiency for 20 wk., starting 60 days after calving, was a requirement of 56.7 lb. of TDN per 100 lb. of 4% FCM, with a standard deviation of 8.2 lb. The range of the individual cows was from 42.0 to 82.7 lb.

Since there is no easy measure of efficiency on the farm, it is of interest to know whether efficiency is closely related to some other variable. The correlations between efficiency and four other variables—4% FCM production, TDN consumption, age, and weight—are given in Table 3. The point of practical

TABLE 3

Correlation between efficiency, FCM production, TDN consumption, age, and weight

	Correlation
FCM and efficiency[a]	−0.71
TDN and efficiency	−0.20
Age and efficiency	+0.12
Weight and efficiency	+0.08

[a] Efficiency is equivalent to: pounds of TDN required to produce 100 lb. FCM (e.g., as FCM increases the pounds of TDN/100 lb. of FCM decrease).

importance is that efficiency is highly related to milk production. The fact that the correlation is negative means that, as efficiency was defined, the more milk produced the lower is the TDN requirement for each 100 lb. of milk. Numerically, this value of −0.73 is almost identical to the 0.75 reported by Hooven and Matthews (12). The correlation between efficiency and weight is very small, if not actually zero. This is in support of the contentions of Brody (3) and Kleiber (14).

The question often arises as to whether early-cut forages are more efficiently used than are late-cut. The results for the data studied are given in Table 4. They show that highly significant reductions occur both in intake

TABLE 4

Effect of date of cutting of forage on efficiency

Cutting date	No. of observations	TDN[a] intake	FCM production	TDN to produce 100 lb. FCM
	(cow-weeks)	*(lb/cow/day)*	*(lb/cow/day)*	*(lb.)*
Early-cut (before June 15)	1,475	23.8[b]	42.4[b]	56.1
Medium-cut (June 15–June 30)	1,380	22.5[b]	39.6[b]	56.8
Late-cut (after July 1)	625	19.2[b]	33.3[b]	57.7

[a] Adjusted for body weight changes.
[b] Significant at 1% level of probability.

and in production, but at about the same rate, so that efficiency does not change. There appeared to be a very slight reduction in the value of a pound of TDN as stage of maturity advanced. For early-, medium-, and late-cut forage, 1 lb. of TDN produced 1.78, 1.76, and 1.73 lb. of FCM, respectively.

Of considerable interest in this study was the fact that cows receiving grass silage consistently produced as much or more milk while consuming less nutrients. Table 5 shows the results of 1,350 cow-weeks, in which early-cut hay (before June 15) was compared with grass silage cut at the same time and from the same field. First to be considered here is the possibility of error in the determinations of the silage dry matter by toluene distillation. It is believed that if error was introduced here it is not large enough to explain the difference in dry-matter intake. The figures for total TDN intake are much closer together, because the cows receiving silage were producing more and were, therefore, given a little more grain (10.6 lb. as compared with 8.4 lb.). However, this extra TDN was charged to them, as indicated in Table 5 under the

TABLE 5

Comparative efficiency of silage and hay for milk production

Roughage	DM intake from forage	Total TDN intake[a]	FCM production	TDN per 100 lb FCM[b]
	(lb/day)	*(lb/day)*	*(lb/day)*	*(lb.)*
Hay	30.4	24.6	41.3	59.6
Silage	26.0	23.5	43.2	54.5

[a] Adjusted for body weight changes.
[b] Differences are significant at 1% level of probability.

column headed Total TDN Intake. Also, there was a very minor adjustment for differences in body weight changes. The cows on silage gained 0.24 lb. and those on hay gained 0.25 lb. a day. For each pound a cow gained, her TDN available for maintenance and production was reduced by 3.53 lb. and for each pound lost the TDN was increased by 2.73 lb., as reported by Knott *et al.* (15).

Expressing the difference between silage and hay in terms of efficiency, it was found that 100 lb. of 4% FCM could be produced with 5 lb. less TDN when silage was the forage source. This difference was consistent in all of the experiments and highly significant ($P < 0.01$). This has been reported earlier by Graves *et al.* (10), Hodgson *et al.* (11), and Nicholson and Parent (17).

Two possible explanations are being proposed which might explain this difference in favor of silage. First, in the ensiling process, lactic acid and other fatty acids are produced by fermentation. After subsequent consumption of silage, these acids may be absorbed directly from the rumen with little or no change. This could represent a saving to the animal in terms of the digestive process, as compared with eating hay. Second, there is evidence presented by Carroll and Hungate (4) and Huffman (13), that the proportions of the volatile fatty acids produced in the rumen vary according to the diet. This was further confirmed by Elliot and Loosli (8). The literature indicates that a ration with silage produces a higher proportion of propionic acid, as compared with acetic, than when hay serves as the forage source. It is a well established fact that the heat increment for propionic acid or lactic acid is small as compared with that for acetic. This, then, could be a plausible explanation why silage may be utilized more efficiently than hay.

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EFFECT OF CONDITION OF ANIMAL ON THE REQUIREMENTS OF ENERGY FOR MAINTENANCE OF THE BOVINE [1,2]

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SUMMARY

Three sets of monozygous bovine animals (two sets of triplet bulls and one set of twin steers) were used to study the relationship between the feed energy required to maintain body weight and the condition (fatness) of the animal. The animals within each set were fed rations with different contents of energy but which were not limiting in their supply of other nutrients. The rations fed caused certain animals of each set to weigh more and be in higher condition than others in the set. Under these circumstances, the TDN required per unit weight for maintenance increased materially as weight and condition increased. Under the circumstance, where the animals of a set were at the same weight but differed in condition, the animal that was fattest required the most TDN per unit weight for maintenance.

The effect of feeding 15 g. thyroprotein to one bull in a set of triplets caused maintenance requirements per unit weight for TDN to be greater than for the bull of the set maintained in very high condition.

It was concluded that maintenance requirements for energy per unit weight increase as condition of the animal increases or as effective quantities of thyroprotein are included in the ration of the bovine animal.

Weight is the only criterion used for determining the maintenance requirements of animals. No consideration is given to condition (degree of fatness). A maintenance ration is the amount of feed required to support an animal which is doing no work and yielding no products. In maintenance trials with mature animals which are kept at constant weight, it is generally assumed that body composition has not changed. Growing animals are considered at maintenance if weight is held constant without considering the changes which take place in body composition.

One of the first to imply that condition influenced maintenance requirements was Haecker (5), who reported that the cost of producing a pound of butter increased when a cow carried an average of 80 lb. more flesh one year than the previous year. Armsby (1) reported that the maintenance requirements of cattle were not proportional to body weight but were approximately proportional to the exposed body surface area. The author hints that condition affects maintenance requirements, by saying that the maintenance requirements of fattening animals increase more rapidly than as a simple function of surface area.

Armsby and Fries (2) found that the maintenance requirement of one steer increased 36% by fattening for 3 mo. during which the live weight increased about 25%. Eckles *et al.* (3) found that very fat calves required nearly 25%

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more energy for maintenance than calves in normal condition at the same weight. McCandlish and Gaessler (6) studied the maintenance requirements of two cows in high condition and later when their condition was reduced. On the average there was a weight decrease of 23% from the high- to the low-condition period. The decrease in the amount of dry matter consumed for maintenance averaged 28.6%.

Trowbridge *et al.* (8) found that the condition of steers had no influence on the cost of maintenance. They observed that fat steers lost considerably in fatness on a maintenance ration. The steers were using up stores of fat from their bodies while at the same time laying down an equivalent weight of nonfat materials. Such an experiment is considerably different from ones using animals that have attained adult form.

It is the purpose of this paper to report the effect of condition (fatness) of animals upon the amount of feed nutrients (TDN) needed to hold weight constant, using monozygous twins and triplet cattle as subjects.

EXPERIMENTAL PROCEDURE

Two sets of monozygous triplet bulls and one set of twin steers were available. The over-all plan was to full-feed certain animals and allow them to grow without any restrictions. Pair-mate animals were fed to restrict growth. Within an experimental group, rations differed only in quantity between animals, with one exception which will be noted.

Animals and their treatment. Triplet, grade, Guernsey bulls, T-15, T-16, and T-17, were born in September, 1946. Each of these bulls received the same amounts of feed until December, 1948. At this time they weighed about 800 lb. and were fed at different levels. T-16 was fed the normal ration, based on Morrison's total digestible nutrient (TDN) requirement for mature bulls in service (7), and was the guide for feeding the two other bulls. T-15 received 30% less and T-17 received 30% more than T-16 from December, 1948, to September, 1952. From September, 1952, to June, 1954, T-15, T-16, and T-17 were maintained, respectively, on 70, 100, and 130% TDN, based on the upper figure of the range in Morrison's standard. These calculations were based on each bull's individual weight. This change was made because a bull could be receiving 70% of the normal bull's ration and actually be receiving over 100% TDN requirements based on his weight. The condition of the bulls during this time was very thin for T-15, fat for T-16, and very fat for T-17. Shortly after this period was completed, T-16 was sacrificed due to an injury which caused paralysis of the hind legs.

The feeding regimes of the high-plane bull and the low-plane bull were reversed, to bring the bulls to a common intermediate weight and thus eliminate the difference in weight as a factor influencing the relation between condition and feed requirement.

In September, 1956, Bulls T-15 and T-17 were at their common intermediate weight and were maintained at these weights until November, 1957, when T-15 died. During this period T-15 was very fat and T-17 very thin.

Identical twin Holstein steers (T-258 and T-259) were fed the same ration until they were 1 yr. old. At this time the weights of the steers were 606 lb. for T-258 and 618 lb. for T-259. The rations were changed so that T-258 would be full-fed and T-259 would receive one-half this amount. At 24 mo. of age T-258 weighed about 1,300 lb. and T-259 about 1,000 lb. At this time a 4-wk. maintenance trial was conducted with a 1-wk. adjustment period.

In June, 1958, one set of 2-yr.-old Holstein bulls, T-233, T-234, and T-235, became available for this experiment. The bulls weighed about 1,450 lb. and had previously received identical rations. The rations were changed so that T-233 received one-half as much as T-234 and T-235. In addition, T-235 was fed 15 g. thyroprotein per day.

Ration weights were recorded daily. A constant ratio of hay to grain was fed during any feeding period and the quantity of feed was all that varied between animals in a set, except for rations fed T-15, T-16, and T-17 before September, 1956. Animals were weighed each month about the same time of day for each set. Animals were housed in separate stalls with access to water, were bedded with wood shavings, and were generally exercised every other day.

RESULTS AND DISCUSSION

Bulls T-15, T-16, and T-17. Bulls T-15, T-16, and T-17 were maintained, respectively, on 70, 100, and 130% TDN, based on the upper figure of the range of Morrison's standard. The average weights for 22 30-day periods were 1,215 ± 9.05 lb. for T-15, 1,584.4 ± 37.1 for T-16, and 1,829 ± 40.1 for T-17. The average rations these bulls received during this time contained 7.95 lb. TDN for T-15, 13.75 lb. TDN for T-16, and 18.92 lb. TDN for T-17. The pounds of TDN required to maintain each 100 lb. of body weight for one day were 0.65 for T-15, 0.87 for T-16, and 1.03 for T-17. As weight and condition increased, the maintenance requirements per unit of body weight increased. It was assumed that increased condition was the factor which increased maintenance requirements, because in feeding standards the maintenance requirements decrease per unit weight as weight increases.

T-15 and T-17 were brought to a common intermediate weight by reversing their planes of nutrition, eliminating weight differences as a factor influencing the relationship between condition and feed requirement.

When the ration of T-17 was reduced from 19 to 13 lb. TDN per day, the bull lost only 15 lb. during the first month. If this had been considered a maintenance trial, the bull would have been assumed to have been maintained, since the weight loss was less than 1%. Apparently, the bull was displacing body fat used for energy requirements with some temporary materials, e.g., water, so that weight loss was low for an initial period of about 1 mo. and quite rapid thereafter.

The bulls had been maintained at their intermediate weights for 59 wk. when the experiment terminated due to the death of T-15. The average weights during this 59-wk. period were 1,483 ± 28.7 lb. for T-15 and 1,484 ± 20.1 lb. for T-17. The average rations received during this time were 13.15 lb. TDN

for T-15 and 9.95 lb. TDN for T-17. This was 32.16% greater TDN for T-15 than for T-17. The amount of TDN required to maintain 100 lb. body weight was 0.88 for T-15 and 0.67 for T-17.

The greatest differences in feed requirements were at the beginning of the experiment, and toward the end of the 59-wk. period they were less. It was unfortunate that T-15 died, since at the end both bulls apparently required the same amount of feed. It looked as if the amount of TDN required to maintain the high condition of T-15 decreased as time went on. Why the maintenance requirement of T-15 decreased with time is impossible to explain. It may have been due to loss of activity with advancing age or some long-time adjustment. From appearance the condition of T-15 was the same during the time the bulls were at their common intermediate weight. Maintenance trials cited in the literature were of much shorter duration and no such trends were reported. When these same bulls were maintained at different weights, no such trend was noted.

Steers T-258 and T-259. At 2 yr. of age the steers were placed on maintenance rations calculated from the formula of Gullickson and Eckles (4). The formula for calculating the TDN required for maintenance is:

$$\text{Pounds TDN required} = 7.925 \frac{(\text{Weight})^{2/3}}{(1{,}000)}$$

The results of this trial indicated that if the average weight was considered the weight maintained, the larger, fatter steer (T-258) consumed 2.4% more TDN, and the thinner, smaller steer (T-259) consumed 2.1% less than the calculated needs. It should be pointed out that these steers were still growing. From the work of Trowbridge (8) it could be assumed that while the fat steer maintained a constant weight, it was most probably using body fat as energy to supplement that in the ration. The results indicated that the thin animal required less than the calculated amount when compared to the fatter animal. Furthermore, it was apparent that the thinner animal expended more energy in movement and, as a consequence, this accentuated the demonstration that fatty tissue either expends more energy per unit mass than lean tissue in maintaining itself, or causes the supporting lean mass to metabolize more rapidly than usual.

Bulls T-233, T-234, and T-235. The bulls weighed about 1,450 lb. when they were first fed on different planes of nutrition. T-233 received 7.75 lb., and T-234 and T-235 received 15.50 lb. TDN daily. After 21 mo. on these rations, T-234 was being maintained at 2,000 lb. in a fat condition while T-233 was maintaining his initial weight of 1,450 lb. The pounds of TDN required to maintain 100 lb. body weight were 0.53 for T-233 and 0.78 for T-234. After 21 mo. T-235, the bull fed thyroprotein, weighed 1,850 lb. Comparing T-235 with T-234, it was apparent that the daily feeding of 15 g. thyroprotein decreased weight gains, probably as a result of increased maintenance requirements. It is interesting to note that the heart rates of T-234 and T-235 were almost identical.

It was noted throughout these experiments that the underfed or thin ani-

mals were considerably more active than their full-fed identical brothers. The underfed or thin animals had dull, lusterless, rough-looking hair coats compared to the slick, shiny hair coats of the full-fed animals.

It was not possible to define in a quantitative manner the condition (fatness) of the animals in this experiment. For this reason it is not reasonable to suggest that consideration be given in feeding standard requirements for differences in condition of animals. It would likely be impractical to define quantitatively the condition of animals, if means to do so were at hand.

It will probably suffice to reiterate that feeding standards are no more than guides by which to help estimate feed requirements wisely. Condition is another factor that is necessary to take into account as one of those things not taken care of by the standard.

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LACTATION STUDIES. II. COMPARISON OF TECHNIQUES TO MEASURE TRUE MILK AND FAT SECRETION RATES IN DAIRY CATTLE AS APPLIED TO BETWEEN-MILKING INTERVALS OF VARIOUS LENGTHS OF TIME[1, 2]

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SUMMARY

Twenty cows were used over a 2-yr. period to compare three techniques of measuring rates of milk and fat secretion. Apparent secretion rates (pounds of milk obtained at normal milking times divided by hours in the between-milking intervals to get pounds per hour) were compared to true secretion rates which were determined by two methods, using oxytocin to obtain complementary milk. Oxytocin was used at four consecutive milkings and at four milkings spaced so as to effect recovery between injections. Three milkings schedules were employed, i.e., (10–14), (11–13), and (12–12). Actual secretion rates were computed and these also were converted to relative rates which expressed the relation between day-time and night-time secretion rates.

The true secretion rate measurement which was most consistent was the one in which oxytocin was injected at consecutive milkings. It was necessary to consider differences in complementary milk left in the udder after milkings, even when a 12–12 hr. milking schedule was used, in order to measure true secretion rates. True secretion rates of milk and fat on a within-interval basis were increased significantly by decreasing the between-milking interval by 4 hr. relative to the other interval in the same day (14 and 30% higher for milk and fat, respectively). The milk and fat secretion rates were higher by 5 and 10%, respectively, during the 11-hr. intervals, as compared to the 13-hr. intervals, but were not statistically different. It was not possible to ascertain whether the 24-hr. rate was affected by changes in milking schedule, because schedule and lactation trend effects were confounded.

It has been noted that as between-milking intervals are lengthened, secretion rates for milk and butterfat are depressed (2, 5, 8). This was thought to be true whether series of consecutive long and short milking intervals or alternate long and short intervals were compared. For example, milking three times per day, as contrasted to twice, results in more and richer milk on a per-day basis (8). In instances in which two or more milking intervals of different durations are used each day, e.g., milking at 5 A.M. and 3 P.M., the milk collected after the shortest interval was richest in fat content, and the yield of

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milk per hour since last milking was greatest (10). An explanation of these phenomena was found in the hypothesis that milk and fat secretion rates are of greatest magnitude directly after milking and thereafter decrease proportionately to elapsed time or build-up of intramammary pressure. Secretion rates of individual between-milking intervals based on normal yields of milk and fat and the time between milkings are not accurate, especially when relatively long and short milking intervals are alternated (4, 12, 13, 14, 15). Complementary milk, obtained after completing the normal milking, was present in considerably larger quantities after long between-milking intervals than after short ones (13). Consequently, a quantity of complementary milk secreted during the long interval was carried over and released with the milk secreted during the short interval, and was thus erroneously credited to the secretion of the short interval (9, 13). The fat secretion rate becomes biased in favor of the short interval to an even greater extent because the carry-over milk is especially rich in butterfat content (9).

To measure true secretion rate, it is necessary to know how much milk was in the udder at the start of the interval and after the milk has been removed by normal milking at the completion of the interval. By knowing how much complementary milk was present at the start and end of milking intervals, and its fat content, an accurate assessment of milk and fat secretion rates may be had, if we can assume that the residual milk left after removing the complementary milk remains constant from one milking to another. There is no method at present by which the residual milk, as distinguished from complementary, can be measured in the living animal. After taking complementary milk into account, Turner (12, 13, 14, 15) and Elliot and Brumby (7) found it impossible to distinguish between hourly secretion rates of milk during milking intervals of various lengths until the milking interval had extended to approximately 20 hr., at which time a depression in secretion rate was noted. The fundamental concept that secretion rate of milk decreases hour by hour from one milking to the next has been seriously challenged by these recent observations, and it becomes important to resolve this discrepancy. As a first step, techniques must be developed which will measure true milk secretion rates accurately over short intervals. This report contains data comparing two techniques in which oxytocin was used to aid in the measurement of true milk and fat secretion rates between milkings, and compares true secretion rates on a within-milking schedule basis, using three different milking schedules.

EXPERIMENTAL PROCEDURE

Data were collected from twelve cows within the University herd during each of 2 yr. Only four of the cows were used in both years. The breeds of the cows involved and the levels of production at the initiation of the trials are shown in Table 1. Three twice-daily milking schedules were employed in both years. The experimental animals were milked in regular sequence within the herd. The starting times for the various milking schedules for the herd were as follows: 1st year—3 A.M.-3 P.M. (12-12), 4 A.M.-3 P.M. (11-13), and 5 A.M.-

TABLE 1

Breed, and initial level of milk production of cows used, by year

First year			Second year		
Cow No.	Breed	Initial level of milk production	Cow No.	Breed	Initial level of milk production
		(lb/day)			*(lb/day)*
1	Holstein	49.7	4	Jersey	50.1
2	Jersey	36.2	5	Jersey	29.7
3	Jersey	48.8	8	Jersey	25.1
4	Jersey	45.2	11	Jersey	41.2
5	Jersey	30.1	13	Holstein	49.8
6	Jersey	40.2	14	Holstein	56.3
7	Holstein	33.8	15	Holstein	42.1
8	Jersey	42.6	16	Holstein	46.7
9	Holstein	34.3	17	Holstein	55.5
10	Jersey	25.9	18	Holstein	41.6
11	Jersey	34.6	19	Jersey	32.8
12	Jersey	38.9	20	Jersey	30.2
Av.		38.4			41.7

3 P.M. (10-14); 2nd year—5:30 AM.–3:30 P.M. (10-14), 4:30 A.M.–3:30 P.M. (11-13), 3:30 A.M.-3:30 P.M. (12-12), 4:30 A.M.-3:30 P.M. (11-13), and 5:30 A.M.-3:30 P.M. (10-14). (The first figure in parentheses indicates interval in hours between morning and evening milking and the second the interval between evening and morning milking.)

The length of time in days each milking schedule was used is shown in Table 2. In both years the schedule listed first had been in use several months before injection procedures were initiated. When the cattle were changed to a new schedule, at least two days were used as a transition period during which time data were not used. Variation in time of use of milking schedules the first year was caused by making changes in milking schedules at times to suit the various people involved and had no bearing on the results as far as can be determined. In the second year the experimental procedures used took 15 days to complete for each milking schedule. The first milking schedule was prolonged unduly (eight days) by illness of the senior author. In the first year, control observations (apparent milk secretion rates) preceded each injection by seven days. It was decided to be desirable to use a seven-day observa-

TABLE 2

Chronological order in which milking schedules were used each year, with the number of days each was used during the measurements of milk and fat secretion rates

Year One		Year Two	
Milking schedules in chronological order of use	Days used	Milking schedules in chronological order of use	Days used
12–12	20	10–14	25
11–13	25	11–13	17
10–14	17	12–12	25
		11–13	7
		10–14	7

tion on the apparent relative secretion rates in the second year. The third milking schedule (12-12) was extended to take additional observations on the apparent secretion rates and then the cows were shifted for a week each to a 11-13- and a 10-14-hr. schedule for the same purpose. In these latter instances, the observations were taken the last five days of the seven-day period. The five-day averages were compared with the two-day averages from the spaced injection series (Table 7) and by T test were not found to be different. On this basis, the series were combined into a weighted seven-day average for use in certain statistical comparisons.

Two experimental procedures were designed to estimate true secretion rates of milk and fat on a within between-milking interval basis as differentiated from apparent secretion rates determined by dividing the yield in pounds obtained at a normal milking by the hours in the between-milking interval (abbreviated henceforth as milking interval). The two methods of measuring true secretion rates were dependent upon the use of intravenously administered oxytocin to eject complementary milk after regular milkings. Complementary milk was removed by hand milking shortly after the normal milk had been been removed from the udder by machine milking and hand stripping. At each milking when oxytocin was used, the normal and complementary milks were weighed and analyzed for fat content by the Babcock Method; weights were recorded of normal milk yields collected between injections of oxytocin. Fifteen units of oxytocin were used for each injection the first year and ten the second.

In the first method used to measure true secretion rates, oxytocin was injected after each of three or four consecutive regular milkings, i.e., three injections only on the first milking schedule the first year. This series will be referred to as the consecutive injection series. It was assumed that each injection of oxytocin emptied the mammary gland to the same degree. Therefore, the total amounts of milk and fat, both normal and complementary together, recovered at successive milkings after the first injection, were assumed to have been secreted during the milking intervals between injections. Half of the cows were injected with oxytocin the first time in a series after morning milkings, the other half after afternoon milkings. The half injected first was switched between morning and afternoon milkings when milking schedules were changed. These milking intervals were included in the series for secretion rate measurements for each cow when four injections were used. Injections of oxytocin at consecutive milkings may cause an above-normal rate of secretion, because the secretion starts with the mammary gland containing less than normal starting amounts of milk.

To overcome this difficulty, a second method was modified after the scheme used by Turner in Australia (15). In this series, termed spaced-injection series, the cow had the opportunity to recover between oxytocin injections and secretion of milk was made against a normal pressure gradient. Four injections were used in this series each time a cow's secretion rate was measured. Oxytocin injections were spaced 48 hr. apart the first year (or occurred at the first

milking following 48 hr. after the last injection when a shift was made from a morning to an evening milking or vice versa). Initially, injections were made at two morning milkings and then at two afternoon milkings. There appeared to be a trend with consecutive injections in the amount of complementary milk recovered. Following this finding, another observation was made at a morning milking after a proper interval, and the last four observations were used in the calculations of the secretion rates. From this time on, two pairs of observations were made in reverse order between morning and evening milkings to balance out treatment and other time-trend effects. Furthermore, the cows were divided into two groups, each of which was started on experimental procedures alternately at morning or afternoon milkings on successive milking schedules. The half which started with injections at morning milkings on a particular milking schedule on this series was opposite to the half which started at morning milkings on the consecutive injection series. During the second year, the minimum interval between injections was raised to 72 hr. on the spaced-injection series.

For the spaced-injection series, milk secretion rates were calculated by adding together the normal and complementary amounts of milk at the milkings at which injections were made, and subtracting from these totals the amounts of complementary milk obtained at milkings taken at other days at the opposite time of the day. This remainder was then divided by the hours in the milking interval involved. Calculations for day and night true and apparent milk secretion rates are illustrated by an actual example taken from the 11-13-hr. milking schedule:

Date	Milking time	Normal milk			Complementary milk		
		(lb.)	*(%B.F.)*	*(lb. fat)*	*(lb.)*	*(%B.F.)*	*(lb. fat)*
May 20	3:30 P.M.	19.7	4.6	.906	2.0	14.0	.280
May 24	4:30 A.M.	21.3	4.1	.873	2.7	13.8	.373
May 27	4:30 A.M.	16.9	3.8	.642	3.0	12.4	.372
May 30	3:30 P.M.	16.5	4.3	.710	1.8	12.2	.220

True day secretion rate for milk equals $\frac{19.7 + 2.0 - 2.7 + 16.5 + 1.8 - 3.0}{22}$ to give pound/hour.

True night secretion rate for milk equals $\frac{21.3 + 2.7 - 2.0 + 16.9 + 3.0 - 1.8}{26}$ to give pound/hour.

The apparent rates for milk would be as follows:

Day rate equals $\frac{19.7 + 16.5}{22}$ to give pound/hour.

Night rate equals $\frac{21.3 + 16.9}{26}$ to give pound/hour.

The spaced-injection series provided two-day averages for both day and night milking intervals for apparent and true milk and fat secretion rates. The fat

secretion rates were calculated in similar manner. During the first year, six cows were used on each injection series and in the second, 12 cows on both series.

For practical reasons, only one milking schedule at a time could be employed. Thus, lactation trend effects on secretion rates are confounded with milking schedule effects. In the first year, the two injection sequences used during milking schedules were conducted independently of one another. In the second year, the consecutive-injection series was followed immediately by the spaced-injection series except on the first schedule, e.g., a lapse of eight days caused by illness of the investigator. In any instance it may be considered, from the lactation standpoint, that the difference in time between injection series was of no consequence in comparing the results of the two methods. Both injection sequences were used once on each cow during each of the three milking schedules. The primary interest was to compare the secretion rates of milk and fat in the two milking intervals occurring in a single day. It was necessary to determine the effect of length of milking interval upon secretion rates on a within-milking schedule basis. To achieve this end, the data for statistical analyses were utilized in two forms: (1) The morning and evening secretion rates of milk and fat for each milking schedule were combined into a relative day secretion rate (r.d.s.r.) expressed mathematically as follows:

$$\text{r.d.s.r. (\%) equals } \frac{\text{day rate minus night rate} \times 100}{\text{night rate}}$$

(2) The data for milk and fat secretion rates were expressed as pounds per hour and utilized as such. Data were treated statistically by methods outlined by Snedecor (11) and Anderson and Bancroft (1). The data used for analysis of variance were individual or mean values from day or night milking intervals by cows on each particular schedule. These data were comprised of different numbers of individual observations, depending upon the variable under study, e.g., one or two milking-interval observations made up the datum for consecutive-injection data for milk and fat, whereas seven observations were available for apparent r.d.s.r. for milk. In certain instances, data for both years were combined but in most instances were analyzed separately. Major efforts were spent in the analysis of the more comprehensive results of the second year, in which the same group of cows was subjected to both measurement techniques.

RESULTS AND DISCUSSION

The first area of interest—the determination of the method which determined true secretion rates most reliably—will be considered first. The techniques used the first year were exploratory in nature and different cows were used with both of the techniques employed. For these reasons, the results from the first year will not be considered in making a comparison of secretion-measuring techniques.

The average values, with standard deviations, for milk and fat r.d.s.r. for the three milking schedules combined, are shown in Table 3. Analysis of data in Table 3 revealed that the error variances for the milk and fat data were

TABLE 3

Mean values for the per cent difference between day and night secretion rates (r.d.s.r.) of milk and fat, and error variances associated with the different techniques used to measure the r.d.s.r. of milk and fat. Twelve cows on three milking schedules. Second year

Method used to measure secretion rate	Relative day secretion rates	
	Milk	Fat
	Mean ± standard deviation (%)	
Control series[a]	+12.3 ± 9.9	+25.5 ± 27.6
Injections at consecutive milkings	+ 7.0 ± 9.0	+12.6 ± 19.2
Injections at spaced milkings	+ 5.1 ± 10.5	+10.6 ± 70.1

[a] Two-day series taken from within the spaced-injection series.

not homogenous. On this basis, separate analyses were made of the milk and fat data. The mean values for fat r.d.s.r. averaged considerably above the related milk values, which is in agreement with others (6, 8, 9, 10, 13, 14).

The error variances for milk r.d.s.r. for the three measuring techniques were all estimates from the same population. Analysis of variance of the r.d.s.r. values from the 12 cows on the three milking schedules, and measured by the three techniques, disclosed no significant differences between the two series of values derived by the two true secretion rate measurement methods, but each was statistically lower than the series of values obtained by the apparent rate measurement method (Table 4, second year results, and Table 4A). Both years gave consistent results on this point (Table 4).

There were highly significant differences in r.d.s.r. in milk due to milking schedules, but a consistent pattern was not apparent in the data at this point. The first year data (4) indicated that schedule effects did not appear to be important. Further evidence on the effect of milking schedules upon r.d.s.r. will be presented to show they are important.

Because of the nonhomogeneity of error variances among the fat data for average r.d.s.r., only the data from the apparent rate method and consecutive-injection rate method were compared by analysis of variance technique (difference in error variances between these two series was barely significant and an analysis of variance was performed without transformation of data). The difference between the mean values due to method of measuring secretion rates in these two series approached significance at the 1% level of probability and milking schedule differences were highly significant. In both respects, these results agree with the data for milk r.d.s.r. (Table 4A). Cow differences in the fat analysis data were not significant, but they proved to be significant at the 5% level of probability in the milk data.

The data from the consecutive injection series contained considerably less error variance than those from the spaced-injection series, even though there were 25% less observations making up the cow mean values. For this reason, and because the consecutive-injection series takes less time, the consecutive-injection series method of determining true secretion rates is favored over the spaced-injection method to measure true secretion rates. It appeared that a series of consecutive injections of oxytocin over such short intervals did not

TABLE 4

Relative day secretion rates (r.d.s.r.)[a] of milk by cows on three milking schedules and measured by three methods. First and second year data

	Milking schedule								
	10–14			11–13			12–12		
	Method of measurement			Method of measurement			Method of measurement		
Cow	a[b]	b[c]	c[d]	a	b	c	a	b	c
	First year results								
1	17.4	.....	−6.8	42.2	.....	9.5	39.3	.....	2.8
2	5.2	.....	4.5	11.5	.....	2.9	6.2	.....	−6.3
3	11.5	.....	−3.0	5.8	.....	6.8	4.0	.....	2.7
4	8.2	.....	−1.9	11.7	.....	1.5	0.0	.....	1.7
5	9.3	.....	0.8	14.0	.....	7.4	2.4	.....	3.6
6	12.2	.....	−1.8	16.2	.....	−9.6	1.2	.....	0.7
7	1.0	8.8	.....	0.7	0.0	.....	−0.4	−12.3	.....
8	−4.8	−2.7	.....	3.0	18.2	.....	−3.2	−3.7	.....
9	−0.8	−6.7	.....	8.5	−1.5	.....	10.6	4.3	.....
10	2.2	−5.3	.....	2.9	−2.8	.....	8.0	−16.2	.....
11	1.7	−9.6	.....	−0.7	−18.0	.....	0.0	−7.7	.....
12	1.9	17.4	.....	4.0	.....	.....	3.5	.....	.....
Average	5.4	0.3	−1.4	10.5	−0.8	3.1	7.1	−7.1	0.9
	Second year results								
4	2.3	15.2	31.8	12.5	9.5	17.1	9.8	14.1	−1.6
5	23.2	17.1	12.9	17.8	−34.2	3.1	23.3	8.7	−5.0
8	13.2	−4.4	33.1	11.7	1.1	10.2	7.6	1.2	−10.2
11	−2.0	0.0	12.3	3.4	−3.0	11.0	5.0	9.5	4.8
13	25.8	30.2	26.2	24.4	17.5	−3.2	4.9	16.5	13.3
14	−4.9	−9.7	3.2	9.0	−10.8	4.1	2.6	−7.1	5.0
15	14.7	3.5	4.0	29.2	1.3	−5.9	13.0	6.3	4.0
16	10.1	2.4	14.2	2.9	−6.0	20.0	13.0	0.8	6.8
17	21.9	25.7	13.3	10.5	3.6	−2.8	4.4	19.1	10.9
18	24.8	27.2	2.3	9.9	13.3	5.3	−2.3	2.5	−6.8
19	14.9	11.6	0.8	6.3	3.8	2.3	22.9	−23.0	−11.8
20	23.3	3.8	18.8	15.7	5.7	−1.7	13.6	8.8	10.5
Average	13.9	10.2	14.4	12.8	0.1	5.0	9.8	4.8	1.7

[a] r.d.s.r. (%) = per cent difference between day and night secretion rates with night rate as base.

[b] Apparent r.d.s.r.—normal yield (pound) divided by time (hours) in milking interval; seven-day averages.

[c] r.d.s.r.—calculated from spaced injection series on true secretion rates, based on two-day averages.

[d] r.d.s.r.—calculated from consecutive-injection series on true secretion rates; one or two milking intervals in each average.

TABLE 4A

Analysis of variance of milk and fat r.d.s.r., considering three measuring techniques for milk and two for fat and three milking schedules with twelve cows

	Degrees of freedom		Mean square	
Source of variance	Milk	Fat	Milk	Fat
Milking schedules (S)	2	2	776**	4,858***
Testing procedure (I)	2	1	515**	2,974*
Cows (C)	11	11	252*	726
S × I	4	2	104	153
S × C	22	22	104	728
I × C	22	11	174*	1,080*
S × I × C (error)	44	22	94	403
Total	107	71		

* Significant difference at 5% level of probability.
** Significant difference at 1% level of probability.
*** Significant difference at 0.1% level of probability.

materially change the milk and fat r.d.s.r. from that observed on the spaced-injection series.

As a second point in these studies, it was of interest to examine the variation normally exhibited by cows in the differences between day secretion and night secretion rates on different milking schedules. The apparent, as well as the true, per cent difference in secretion rates between day and night rates (r.d.s.r.) for milk are shown in Table 4. The data presented under the apparent r.d.s.r. are averages compiled from observations from seven normal days of milking on the various milking schedules. It is quite obvious that these values vary considerably from cow to cow, schedule to schedule, and year to year. The data for apparent r.d.s.r. for milk for both years were subjected to analysis of variance technique and the results are shown in Tables 5 and 6. In the

TABLE 5

Analysis of variance of the apparent r.d.s.r. for milk. Total data

Source of variance	Degrees of freedom	Mean square
Years (Y)	1	460***
Cows in years	22	199***
Milking schedule (S)	2	73
S × Y	2	56
Remainder	44	34
Total	71	

*** Significant difference at 0.1% level of probability.

TABLE 6

Analysis of variance of apparent r.d.s.r. for milk of four cows used both years

Source of variance	Degrees of freedom	Mean square
Years (Y)	1	310***
Cows (C)	3	207***
Milking schedules (S)	2	28
C × Y	3	61
C × S	6	10
S × Y	2	30
Remainder	6	20
Total	23	

*** Significant difference at 0.1% level of probability.

analysis of Table 5, it was not possible to clear year effects of cow effects, as most of the cows were used only a single year. Table 6 shows an analysis of data taken from the four cows common to both years. It is clear that cows vary from one to another in the same year, but also may vary in themselves from one year to the next. Reports in the literature (3, 6) show that the apparent r.d.s.r. for milk decreased with advance in lactation (decline in milk production). Level of milk production apparently was not the explanation for differences between cows or even in the same cow from one year to the next, e.g., Jersey No. 8 had a higher r.d.s.r. in the second year, compared to the first, at which time she was producing less milk than in the first year. Although the averages for the second year in Table 6 are highly significantly higher for apparent milk r.d.s.r., there appears to be no logical explanation for this that

can be advanced. In neither set of data did there appear to be a pattern of change in apparent r.d.s.r. for milk due to milking schedules. In the analyses presented in Tables 5 and 6 schedule effects are not confounded with lactation trends. This situation holds only for data combined from both years.

In order to have a seven-day series on the apparent rate of secretion of milk in the second year, it was necessary to combine data taken at two different times. A two-day series was taken at the times of injections in the spaced-injection series. At later dates, observations were made of normal milkings to compile a five-day series. The average values with standard deviations for these two series are presented in Table 7. The mean values of the r.d.s.r. for

TABLE 7

Average values with related standard deviations of apparent r.d.s.r. taken in a two-day series and a five-day series, with the interval of time between finish of two-day series and start of five-day series

Milking schedule	Two-day series	Five-day series	Days between two series
	Average apparent r.d.s.r. (%) ± standard deviation		
10–14	15.5 ± 12.9	13.3 ± 11.3	51
11–13	8.5 ± 8.0	14.4 ± 10.4	34
12–12	13.1 ± 13.1	8.5 ± 8.2	2

the two series were not statistically different from each other. The longest interval of time between the end of the two-day series and the beginning of the five-day series was 51 days (Table 2, 10-14 hr. schedules). During this time, the average daily production of cows dropped from 43.1 to 33.8 lb. per day. In this series of observations, there was no evidence that the milk r.d.s.r. decreased with a drop in milk production or advance in lactation. No further effort was spent to pursue this point. Obviously, the data presented here can not be considered conclusive, because of the small numbers and great variability.

Variances of the apparent r.d.s.r. for milk and fat probably are associated with the phenomena of milk ejection (both inherent ability to eject milk and environmental influences which affect the ability to eject milk). The final answer to the variance exhibited in the apparent r.d.s.r. must await further research effort.

The final phase of the work was concerned in making a detailed comparison of the apparent and true milk and fat secretion rates and r.d.s.r. from selected data. The apparent r.d.s.r. was comprised of the combined two- and five-day series for milk only. The average r.d.s.r. values for milk and fat for each milking schedule are outlined in Table 8. Analysis of variance of the data (Table 8A) revealed highly significant differences among milking schedules, showing a very definite decrease in r.d.s.r. as the day milking interval increased, i.e., divergence of the two daily intervals decreased. In the previous three-way comparison for milk, the schedule effects also were highly significant (Table 4A), but did not fall into a regular pattern. The previous difference between the two methods of making measurements was substantiated. In the later analyses, cow differences were nonsignificant. The differences between the data

TABLE 8

Mean values of the per cent difference between day and night secretion rates (r.d.s.r.) on three different milking schedules, measured by two schemes for milk and fat. Twelve cows, second year

Means to measure secretion rates	Milking schedules					
	10–14		11–13		12–12	
	Milk	Fat	Milk	Fat	Milk	Fat
	(%)					
Control series—apparent secretion rates[a]	+13.9	+37.5	+12.8	+25.1	+9.8	+14.0
Experimental series—true secretion rates[b]	+14.4	+30.4	+ 5.0	+10.5	+1.7	− 2.8

[a] Milk, seven-day averages; fat, two-day averages.
[b] Milk and fat data based on only one or two milking intervals for both of the two daily milking intervals for each cow during the consecutive-injection series.

TABLE 8A

Analysis of variance of milk r.d.s.r. considering three milking schedules and two measurements of secretion rates with 12 cows

Source of variance	Degrees of freedom	Mean squares
Milking schedules (S)	2	437**
Testing procedure (I)	1	481**
Cows (C)	11	82
S × I	2	143
S × C	22	45
C × I	11	140
S × I × C (error)	22	96
Total	71	

** Significant difference at 1% level of probability.

in Table 8 and the data previously discussed were due to two factors: (1) removal of the heterogeneous data from the spaced-injection series, and (2) inclusion of additional observations on the apparent r.d.s.r. for milk, which caused these data to conform to the pattern already apparent from the consecutive-injection series.

The data presented in Table 8 support the findings of Turner (13), that it is necessary to consider the milk left in the udder at the start and finish of a milking interval to accurately assess secretion rate. However, data in Table 8 do not substantiate the hypothesis of Turner and others (7, 13, 14, 15), that milk and fat secretion rates are not affected by increases in milking intervals until rather long intervals are employed, e.g., 20 hr. Considering only the oxytocin injection series (true secretion rates), the r.d.s.r. for milk and fat increases as the daily milking intervals become more divergent. The value for r.d.s.r. (14.4%) shown under the 10-14-hr. schedule for milk is significantly different from zero. The other two figures are not, but the figure under the 11-13-hr. schedule fits a trend away from zero on the 12-12-hr. schedule. The data for the first year (4) did not fit the pattern exhibited by the data from the second year. The second year results, based on superior numbers and less variability of the data, would appear to be the more reliable, and support the

hypothesis that secretion rates of milk and fat are definitely decreased as the milking intervals are increased by 2 or 4 hr. relative to the other milking interval in the same day. The data for absolute rates of milk secretion were not as extensive as the milk r.d.s.r., because the two- and five-day series could not be combined, as it is well known that daily secretion rate is affected by advance of the lactation period. The data for milk secretion rate was more extensive than the fat secretion rate. Data from two pre-experimental days were combined with the normal data taken from within the spaced-injection series to give a four-day series on normal secretion rates for milk. The mean values for milk and fat secretion rates are shown in Table 9. Table 9A contains pertinent data of the analyses of variance. A multiple range test applied to differences of the means within a milking schedule, by method of measurement, disclosed that differences between day and night rates were not significant for true secretion rates for milk or fat on the 12-12-hr. schedule, increased on the 11-13 schedule but were not significant, and became significant on the 10-14-hr.

TABLE 9

Mean values of milk and fat secretion rates (pound/hour) for day and night milking intervals on three different milking schedules. Comparison of apparent rates with true secretion rates

Milking schedules		(10–14)[a]		(11–13)		(12–12)	
Milking interval within schedule		Day	Night	Day	Night	Day	Night
Apparent secretion rate *(lb/hr)*[b]	Milk	1.94	1.70	1.77	1.64	1.62	1.45
	Fat	0.0905	0.0659	0.0886	0.0757	0.0727	0.0664
True secretion rate *(lb/hr)*[c]	Milk	1.91	1.68	1.76	1.68	1.59	1.57
	Fat	0.0850	0.0655	0.0766	0.0695	0.0609	0.0638

[a] First figure represents hours from morning to evening milking; second, evening to morning.
[b] Normal milkings from spaced-injection series.
[c] Consecutive-injection series.

TABLE 9A

Analysis of variance of rate of milk and fat secretion rates (pound/hour) as measured by two methods on three milking schedules using 12 cows

Source of variance	Degrees of freedom	Mean squares	
		Milk	Fat
Milking schedule (S)	2	0.6640***	0.00202***
Method of measuring (I)	1	0.0245	0.00149**
Time (T)	1	0.4994***	0.00455***
Cows (C)	11	1.2434***	0.00339***
S × I	2	0.0086	0.00004
S × T	2	0.0846**	0.00126***
S × C	22	0.0524***	0.00017
I × T	1	0.0008	0.00040
I × C	11	0.0108	0.00074***
T × C	11	0.0112	0.00014
T × S × I	2	0.0288	0.00009
Remainder	77	0.0129	0.00016
Total	143		

** Significant difference at 1% level of probability.
*** Significant difference at 0.1% level of probability.

schedule. These data corroborate the r.d.s.r. data showing that as the night milking intervals increased relative to the day intervals, the differences between day and night secretion rates progressively increased.

CONCLUSIONS

On the basis of error variance, the method of determining true milk and fat secretion rates which was most reliable was the one in which oxytocin was used at consecutive milkings.

It is necessary to consider the complementary milk in the cow's udder before and after a milking interval, to estimate accurately intra-interval secretion rates of milk and fat.

There is error in estimating intra-interval secretion rates from yields, even when equal intervals are concerned, but the error increases as the between-milking intervals become more divergent.

True milk and fat secretion rates on an intra-interval basis become more divergent as the between-milking intervals become more divergent, showing that time or accumulation of milk in the udder depresses secretion rates; e.g., comparing a 10- and 14-hr. interval from the same day.

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CHANGES IN THE TISSUE AND VOLUME OF THE STOMACHS OF CALVES FOLLOWING THE REMOVAL OF DRY FEED OR CONSUMPTION OF INERT BULK

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SUMMARY

Twelve Holstein heifer calves at 16 and 38 wk. of age were sacrificed after a growth study in which six had voluntarily consumed a high-concentrate diet and six a high-roughage diet. Ratios of hay to concentrate were 9:1 and 1:9. Four bull calves, two fed only milk and two fed milk while consuming shavings, also were studied. The objectives were to observe the effects of continuous consumption of a high-roughage, a high-concentrate, and an inert bulk diet on stomach muscle, mucosa, and volume, and to test the effect on stomach anatomy of reversing 16-wk.-old calves from the above ratios of hay to concentrate back to milk.

Rumen papillae disappeared in the calves reversed to milk diets. The rumen collapsed around less fill, but maintained the same potential (water-filled) volume after 18 wk. of milk feeding, as had existed in calves of the same age on dry feed slaughtered at the beginning of the reversal period. This observation was interpreted as indicating a stasis, and not retrogression of reticulo-rumen volume. The amount of muscular tissue (fat-free dry) was less in the reversal calves, but the effect was not statistically significant. A stasis in omasal tissue growth occurred, and the abomasum continued to grow at the same rate as body size.

Mucosal and muscular growth were independent. This was evidenced in three ways. (1) Reticulo-rumen mucosa retrogressed at a more rapid rate than muscle. (2) Muscular development was extensive in the absence of mucosal development in the calves consuming shavings. (3) Compared to muscle, well-developed mucosa contained lower nitrogen percentages when expressed on a fat-free dry matter basis (13.3 and 15.1%, respectively).

Rumen papillae length was greater in the faster-gaining (high-concentrate) calves. Apparently, both rapid growth and papillary development were due to the higher energy levels consumed by these animals.

The anatomical development of the ruminant stomach is not a simple enlargement of the organ as the animal grows but is markedly affected by diet (3, 7, 8, 9, 13, 16, 17, 21). Three structural entities may change, viz., capacity, mucosal tissue (papillae), and muscular tissue. Previous studies showed that at the early ages the rumens of milk-fed calves exhibit less capacity and papillary development than those consuming hay and concentrate. Recently, it has been shown that the papillae grow in response to the fermentation end products, especially butyrate and propionate (8, 9, 16). In these past studies, however, no consideration was given to muscle development. Because of its importance in ruminal motility, the development of this tissue was deemed worthy of investigation.

Preliminary observations on mature sheep in this laboratory revealed no papillae in one animal which had been fasted for 30 days. This observation gave rise to additional questions regarding the changes in rumen capacity and

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muscle which might occur if dry feed were removed from the diet. The effect of replacing a diet of hay and concentrate with milk was thus examined.

It has been shown (9) that inert bulk (plastic sponges or nylon bristles) does not result in the development of papillae, and causes no marked volume development of the reticulo-rumen. In the course of the present studies an opportunity arose to further examine the effect of inert bulk on rumen structure.

Because the rumen is so important in the nutrition of the animal and is distinctive in its structure, and because Sinclair and Kunkel (17) have observed a limited correlation between rumen papillary length and average daily gain by lambs, rumen development criteria which might be related to the growth of the calves also were sought.

EXPERIMENTAL PROCEDURE

Twelve heifer and four bull calves were used. The heifers constituted the basic experimental group and the bulls were used for supplementary observations. For 7 wk. prior to initiating the experiments the growth and feed intakes of the heifers were as follows:

Six of the heifers had voluntarily eaten 90% of their total dry feed intake in the form of concentrate (a situation which was seen in over 80% of 300 calves recently studied at this station). The six other heifers had voluntarily consumed about 75% of their dry feed as hay. Gains for the two groups during the growth study were 1.1 and 0.67 lb. per day, respectively. The calves in the high-hay group were thin, pot-bellied, and unthrifty. Both groups had consumed 40 lb. of milk replacer, equivalent in energy content to approximately 200 lb. of whole milk (11).

The formula of the starter and the proximate composition of the hay and starter appear in a footnote to Table 1. The hay was a good-quality, second-cutting mixture of grass and alfalfa and was chopped into 4-in. lengths.

The calves were placed on the current experiment at 7 wk. of age and offered the same feeds as in the growth study. From 7 to 10 wk. of age the proportion of hay fed to the high-hay group was gradually increased to 90%. The high-concentrate group was maintained on a diet of 90% concentrate during this period. From 10 to 12 wk. of age feed intakes for both groups were increased and equalized until they received 5.5 lb. of total feed per day for the period of 12 to 16 wk. This was all the high-hay group would consume. At 16 wk. of age two calves from each group were slaughtered and detailed observations made on the anatomy of their digestive tracts. At this same age two others from each group were reversed to an ad libitum milk diet and housed on wooden platforms without bedding. They were kept on this diet for a maximum of 22 wk.[1] The remaining two calves on each dry feed regime were retained on their respective hay-to-concentrate ratios to 38 wk. of age. At this age all remaining dry feed and reversal calves were slaughtered except as noted in Footnote 1. During this latter period weights of feed intake were not kept constant among

[1] Two reversal calves were slaughtered (after 14 and 17 wk. on treatment) due to injury resulting from falling in their stalls.

TABLE 1

Empty body weights, ages at slaughter, and feed intakes of calves immediately before slaughter[a]

Treatment	Age at slaughter	Empty body weight	Feed intake per day[b]	
			Hay[f]	Conc.[e, f]
	(wk.)	*(kg.)*	*(lb.)*	
90% Concentrate	16	86	0.5	5.0
90% Hay	16	58	5.0	0.5
90% Concentrate	38	157	0.6	6.9
90% Hay	38	111	10.0	1.0
Reversal from 90% concentrate	33	145	[c]	[c]
Reversal from 90% hay	35	125	[c]	[c]
Milk	27	171	[c]	[c]
Shavings	47	89	[d]	[d]

[a] Two calves per treatment.
[b] Last 4 wk. before slaughter.
[c] Milk ad libitum.
[d] Milk at maintenance level.
[e] Formula in percentage: wheat bran, 10; crushed oats, 17.5; coarse cracked corn, 17.5; linseed oil meal, 10.0; soybean oil meal (44%), 18.0; alfalfa meal, 7.0; corn distillers solubles, 5.0; dried skimmilk, 5.0; cane molasses, 8.0; $CaCO_3$, 0.5; Ca_2PO_4, 0.5; iodized salt, 0.5; stabilized vitamin A, 0.2; antibiotic feed supplement, 0.15; irradiated yeast, 0.025; cobalt, 2 g/ton.
[f] Proximate analysis in percentage: hay; dry matter, 90.1; crude protein, 15.7; ether extract, 2.3; ash, 5.8; crude fiber, 27.8; NFE, 38.5. Concentrate; dry matter, 90.6; crude protein, 22.3; ether extract, 2.4; ash, 12.9; crude fiber, 8.1; NFE, 44.9.

the calves on dry feed, as was done from 12 to 16 wk. of age, but estimated net energy intakes were kept as close as possible to the calculated intakes (1, 2, 15) of their group-mates which had been reversed to milk.

Two bull calves received whole milk ad libitum supplemented with trace minerals and vitamin D from birth to 27 wk. of age and were housed on wooden platforms without bedding and served as negative controls. These bulls were slaughtered at a younger age than the other calves because they had exceeded the weight of the largest heifers. The two other bull calves were bedded on shavings which they consumed freely from 1 mo. of age, and were fed filled milk supplemented with vitamins and minerals slightly above maintenance for a period of 11 mo., as part of another experiment. They were slaughtered in order to measure the influence of such an inert bulk on rumen structure.

Before slaughter (approximately 4 hr. after feeding) all animals were anesthetized intravenously with pentabarbital sodium. The pylorus and esophagus were exteriorized and ligated with cotton cord, and the animals immediately hoisted by the hocks and bled. The digestive tract was removed as one unit, its volume measured by water displacement (8), then separated into the reticulo-rumen, omasum, abomasum, and intestines. The volume of each of these components was then measured in the same manner. Volumes of the reticulo-rumen and abomasum with 1.5 in. of overpressure [2] also were recorded. Ingesta weight was determined by weighing each compartment full and empty. This weight

[2] Empty organ was floated in water, a ¾-in. glass tube inserted into the esophagus of the rumen or pylorus of the abomasum. These compartments were then filled with water to a level of 1½ in. above the water level in the tank, as measured in the glass tube.

was subtracted from each animal's live weight at slaughter, giving the empty body weight (EBW). The volume of the ingesta was measured by pressing the liquor out of the solids with a lard press until the liquor ceased to flow. The solids were then tamped into a cream can and the volume occupied was measured by replacing them with water. The stomach compartments were photographed and separated into their mucosal and muscle layers. Care was taken to keep the mucosa as free as possible of muscle fibers. The stomach tissues were then dried for 24 hr. under vacuum in a Stokes Desivac at 110° F. and ground through the 2-mm. sieve of a Wiley Mill. A 3- to 5-g. sample was redried and extracted for 48 hr. with anhydrous diethylether, to calculate the total fat-free dry weight of the tissue. The Kjeldahl procedure was used to determine the nitrogen content of the tissue.

Papillary length was measured as follows on tissue slices removed from the dorsal cranial blind sac of the rumen, and fixed in Bouin's solution. Several cuts were made in each tissue slice by slipping a pair of surgical scissors in among the papillae at their base, thus exposing 20 to 30 papillae along the cut. These remained moist and were measured by placing a millimeter rule next to them while gently extending each papilla to full length along the rule. Thirty to 40 papillae were measured at random from each calf (150 papillae per treatment group).

Statistically significant differences were located by an analysis of variance and a sequential test (19) for comparisons of treatments.

It is difficult to decide which is the most satisfactory way to express the capacity and total tissue of the stomachs of ruminants of various ages and weights. All previous publications from this laboratory have expressed rumen capacity and tissue growth per unit of ingesta-free (empty body) weight (8, 9, 16, 21). This technique eliminated the obvious variable of gastro-intestinal fill and tended to standardize the data obtained from animals of different sizes. It has been a useful technique, thus far, in our attempts to ascertain basic principles of rumen development. In the current study, however, in which a possible retrogression of the ruminant stomach was under consideration, we have examined the absolute values to obtain evidence of a reduction in stomach capacity or tissue following a substitution of milk for dry feed. A decline in tissue weight or stomach size of the reversal calves when they were compared to their pair-mates which had been slaughtered at 16 wk. of age (the age when the reversal calves were replaced on milk) would suggest retrogression. Little change would mean a stasis of development and an increase would mean continued growth of the stomach during the milk feeding (reversal) period.

A further attempt has been made to describe the appearance of the rumen wall by calculating the amount of fat-free mucosa and muscle per square decimeter (g/dem^2) of surface. This calculation assumed the reticulo-rumen to be a sphere. Using the water-filled volume and total fat-free dry weight of the muscle and mucosal tissue, the data in Table 2 were derived. While it is recognized that the reticulo-rumen is not absolutely spherical, the divergence from it is similar for all calves and, therefore, treatment differences should retain the same relative position without bias.

TABLE 2
Grams fat-free dry tissue per square decimeter of reticulo-rumen surface

Treatment	Age at slaughter	No. of calves	Mucosa[a]	Muscle[b]
	(wk.)		*(gm/dcm²)*	
90% Concentrate	16	2	3.00	2.33
	38	2	1.59	1.71
		Average 4	2.30	2.02
90% Hay	16	2	1.97	2.50
	38	2	2.07	2.66
		Average 4	2.02	2.58
Reversal from concentrate	33	1	0.83	1.45
Reversal from hay	35	2	1.14	2.08
		Average 3	1.04	1.85
Shavings	47	2	0.85	2.28
Milk	27	2	0.61	1.36
S.D.			0.25	0.23
C.V.			16%	11%

[a] Average 90% hay, average 90% concentrate > average reversal, shavings, milk ($P < 0.05$).
[b] Average 90% hay, average 90% concentrate, average reversal, shavings > milk ($P < 0.05$).

For purposes of reference, Table 1 includes the empty body weights, ages at slaughter, and feed intakes of all animals during the experimental period.

RESULTS AND DISCUSSION

Retrogression of rumen structures. The most striking observation after the reversal to a milk diet was the absence of rumen papillae at slaughter 18 wk. later. This result is demonstrated clearly in Figure 1, which shows photographs of the papillae (reduced to 0.94 of actual) of the ventral-most portion of the dorsal-cranial sac. There were only papillary buds on the mucosa of calves fed milk continuously, whereas the papillae of the reversal calves were of the same length but filiform in shape, indicating an incomplete retrogression. In contrast to the reversal and milk diets, the papillae length of the high-hay calves averaged 4.1 mm., whereas those of the high-concentrate group averaged 7.5 mm. The shapes of the papillae on the hay and concentrate diets were uniform in each calf, but there was no consistent pattern that could be associated with treatment. Three main shapes were observed: spade-shaped, triangular, and hair-like. Papillae were practically absent on the dorsal surface of this organ and extremely dense in the area of the reticular fold, the area from which the photographs in Figure 1 were taken.

Table 2 quantitatively describes the lack of rumen mucosa in the reversal calves. The rumen musculature appears to have retrogressed also in these calves, although the data are statistically significant only when one compares them to the high-hay group. These data indicate that the retrogression of the reticulo-rumen which resulted from re-placement on milk after consuming hay and grain was not complete. Whether this was due to too short an experimental period or to a certain degree of permanence of the developed reticulo-rumen must await further experimentation. Data from the calves on shavings and

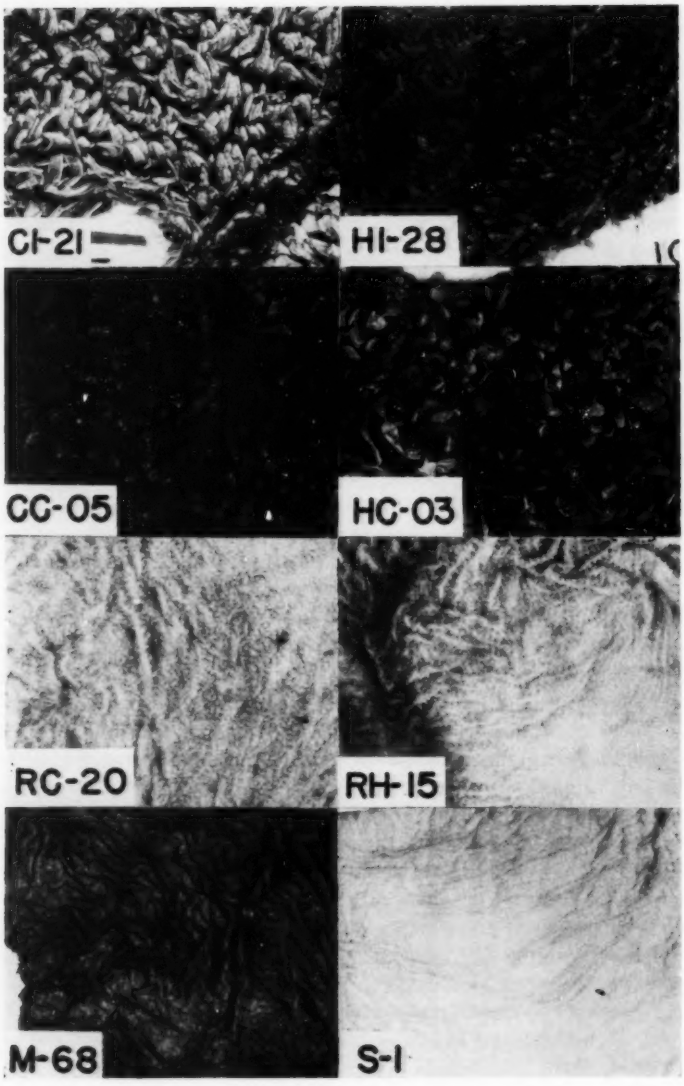

FIG. 1. The rumen mucosas of one-half of the experimental animals. The other replicate gave the same results (11). CI-21—High concentrate, 16 wk.; CC-05—High concentrate, 37 wk.; RC-20—High concentrate for 16 wk., followed by milk for 18 wk.; M-68—Milk ad libitum for 27 wk.; HI-28—High hay, 16 wk.; HC-03—High hay, 37 wk.; RH-15—High hay, 16 wk., followed by milk for 18 wk.; S-1—Milk at maintenance and shavings ad libitum.

continuous-milk indicate that no papillary development took place on either diet, but that considerable muscular development occurred on shavings.

In the authors' opinion, the expression of the data as it appears in Table 2 is the best way we know to eliminate the effect of age and body weight in evaluating rumen tissue development. The age and weight of the continuous-milk and shavings calves diverged far enough from the basic 12-calf group to give little meaning to comparisons on the basis of total stomach tissue and volume (Tables 3 and 4). Therefore, Tables 3 and 4 include only the data from the 12 heifers.

TABLE 3

Volume in liters of the full stomach compartments under conditions of natural fill[a] and standard pressure[b]

Treatment	No. of calves	Reticulo-rumen (Natural fill)[a]	Reticulo-rumen (Standard)[b]	Omasum (Natural fill)[a]	Abomasum (Standard)[b]
1. 90% Concentrate, 16 wk.	2	22	27	1.8	3.3
2. 90% Hay, 16 wk.	2	24	34	2.4	2.6
3. Reversal, 16-34 wk.	3	12	30	0.4	3.3
4. 90% Concentrate, 38 wk.	2	50	66	2.9	5.5
5. 90% Hay, 38 wk.	2	54	67	4.8	3.2
S.D.		±2.1	±4.5	±1.11	±0.91
C.V.		7.5%	11%	50%	25%
Significant differences ($P < 0.05$)		1,2>3 1,2,3<4,5	1,2,3<4,5	1,2,3,4<5	1,2,3,5<4

[a] Water displaced by organ with natural fill immediately after removal from the animal at slaughter.

[b] Internal pressure of 1.2 in. of water (see footnote 1).

TABLE 4

Total fat-free dry tissue of the stomach of calves fed high-hay and high-concentrate diets compared to those reversed back to milk at 16 wk. of age

Treatment	No. of calves	Total stomach	Reticulo-rumen	Omasum	Abomasum
		(g.)			
1. 90% Concentrate, 16 wk.	2	310	220	51	39
2. 90% Hay, 16 wk.	2	286	190	61	34
3. Reversal, 16-34 wk.	4	270	164	42	63
4. 90% Concentrate, 38 wk.	2	465	317	91	62
5. 90% Hay, 38 wk.	2	563	387	128	48
S.D.		±27	±22	±14	±7
C.V.		6.8%	8.4%	18%	12%
Significant differences ($P < 0.05$)		1,2,3<4,5 4<5	1,2,3<4,5 1>3 4<5	1,2,3<4,5 4<5	1,2<3,4,5 3,4>5

In Table 3 the volumes of the various stomach compartments have been summarized. As removed from the carcass, the reticulo-rumens of the reversal group were significantly smaller than all others, reflecting a smaller amount of fill. When the reticulo-rumens were filled to a standard pressure, however, they had the same volume as those calves on hay and grain slaughtered at 16 wk., but significantly less than those fed dry feed and slaughtered at 37 wk.

of age. This observation suggests that there was a stasis of growth in capacity between 16 and 37 wk. for those calves reversed to a milk diet and that the calves had in effect grown around the reticulo-rumen. While the rumens had collapsed around their reduced fill, no reduction in their potential volume (water-filled) occurred.

Reticulo-rumen volumes reported up to now from this laboratory have been the result of water-filling this organ to 1.2 or 2.0 in. of overpressure (8, 9, 11, 16, 21). Flatt *et al.* (9), using calves on natural hay and grain diets, compared in vitro volumes to volumes obtained in vivo from the same animals. They found that 1.2 in. of overpressure in vitro gave the same volumes as those obtained in vivo. In the present study (Table 5), the volume of the reticulo-

TABLE 5

Interior volume of the reticulo-rumen,[a] volume of the rumen contents, per cent filled of the reticulo-rumen, and density of the rumen contents[b]

Treatment	Interior volume reticulo-rumen	Volume contents	Per cent filled	Weight, contents	Density
	(liters)			*(kg.)*	*(kg/l)*
90% Concentrate, 16 wk.	19.3	10.2	54	11.0	1.05
90% Hay, 16 wk.	22.5	14.3	62	13.5	0.95
90% Concentrate, 38 wk.	45.0	23.8	52	21.7	0.95
90% Hay, 38 wk.	50.0	26.6	55	25.4	0.95
Shavings	26.6	26.5	99	26.9	1.00

[a] Volume-as-removed minus the volume of the fresh rumen tissue.
[b] Two animals per group.

rumen, water-filled, exceeded that same compartment with natural fill (volume measured immediately upon removal from the animal). The extent of this difference in volume varied. The reticulo-rumens of the high-hay and the high-concentrate groups, water-filled, were 30% larger than the same organs with natural fill. In the milk-fed group the water-filled volumes were 2.5 to 2.8 times as large. The reticulo-rumens of the shavings group, when filled with water, showed an 80% expansion. These data agree with the recent report of Smith (18), that the rumen volume of the milk-fed calf is largely unused. From these comparisons it is clear that the rumen volume resulting from water-filling is the maximum obtainable without forcefully expanding the rumen. Such volumes probably do not occur in the live animal, because the size of the abdominal cavity is limiting. Their chief usefulness is their uniformity, giving a basis for comparing diets. In milk-fed animals, where little natural fill is present in the reticulo-rumen, a standard value such as this is particularly needed if one is to compare their rumen volumes to calves which have been consuming dry feed.

The volumes of the omasum in the reversal animals were not significantly different from those of animals on dry feed sacrificed at 16 wk., but were significantly smaller than the 37-wk.-old calves on hay. Small treatment groups and the high coefficient of variation (50%) made what appear to be large

average differences, nonsignificant statistically. The abomasal volumes showed no retrogression.

Table 4 summarizes the total fat-free dry tissue of the stomach compartments. These values are affected both by the surface area and by the tissue thickness of the stomachs. These two variables can either add to or cancel each other. In terms of total stomach tissue, reticulo-rumen tissue, and omasal tissue, the reversal calves showed significantly less development than the calves on dry feed slaughtered at 37 wk. When compared to the calves on dry feed slaughtered at 16 wk., the only significant difference was between the reversal and the high-concentrate groups. This difference was to be expected, since the disappearance of mucosa is the most striking feature of the retrogression. Further, the papillae of the calves fed the high-concentrate diet were longer and would thus cause a greater loss of tissue weight when they disappeared.

Omasal tissue remained almost as heavy in the reversal calves as it was in the dry feed group at 16 wk., indicating a stasis of growth. Thus, the smaller average omasal volume in Table 3 must be a result of a reduced fill.

The weight of abomasal tissue clearly shows that this organ did not retrogress but continued to grow in the reversal calves at a rate equal to the calves fed dry feed.

The data for the calves which consumed limited amounts of filled milk and shavings, while not a part of the original experiment, have been included to demonstrate the effect of a large amount of heavy bulk (27 kg., Table 5) on the stomach development. The calves were considerably older than the others and had grown slowly because of their minimal diet. They were, however, apparently normal otherwise. In Figure 1 it is clear that no papillae developed on this regime and in Table 2 the amount of mucosa per unit area is low. This was to be expected, since it has been demonstrated previously (9, 16, 21) that nylon bristles or plastic sponges failed to elicit papillary growth. At slaughter the rumens of the calves on shavings had no active fermentation, as indicated by the absence of any gas pocket (Table 5) and lack of normal rumen odor or pH (pH was 7.2). The rumens of these calves are additional evidence that papillae grow only when stimulated by the end products of an active fermentation.

Independence of muscle and mucosal growth. It is of interest that the muscular tissue appears to have retrogressed at a slower rate than the mucosa. This suggests that these two tissues are influenced independently by diet and fill (Table 2).

The extensive muscular growth by the calves on shavings in the absence of papillae (Table 2) further indicates that muscular and mucosal growth occur independently. A reasonable explanation for the extensive muscle development in these calves appears to lie in the greater weight of residual contents in the rumens of these calves when compared to those on milk (Table 5), the rumen muscle having developed as the result of constantly moving and mixing these contents. Further evidence for this independence is seen when the compositions of these tissues are examined. The nitrogen percentage (fat-free dry

matter basis) of the well-developed mucosas (papillae of 4.1 to 7.5 mm. in length) was markedly and consistently below that of the mucosas with papillae less than 1 mm. in length ($13.3 \pm 0.2\%$ vs. $16.1 \pm 0.2\%$). This reduction in nitrogen could occur in either the epithelium or the collagenous connective tissue which forms the base of the mucosa and the core of the papillae. Hawk *et al.* (12) report the nitrogen percentage in the collagen of beef hide as 18%. If one assumes that the collagenous core of the papilla makes up about one-third of its structure (4) and is 18% nitrogen, one can calculate that about 11% nitrogen is necessary in the epithelium to yield the 13.3% observed in the present study. In contrast, the muscular tissue of all compartments of the stomach for all diets averaged $15.1 \pm 0.2\%$ nitrogen. The ether extract percentage in the mucosa was relatively constant and ranged from an average of 5% in the concentrate and hay groups to 8% in the shavings, milk, and reversal groups. Habel (10) has observed fat histologically in the epithelium of the rumens of calves. In contrast to the mucosa, the fat percentage in the muscle tissue (on a dry basis) ranged from 4% in the calves on shavings to 60% in the milk group, following the plane of nutrition closely.

The independent growth of reticulo-rumen muscle and mucosa observed is consistent with the fact that the mucosa is a connective tissue, epithelial-complex (4), which differs greatly from muscle in its structure, cell type, and histogenesis (14).

Fill as a proportion of body weight. The contents of the digestive tract expressed as a percentage of the live weight were $15 \pm 2.4\%$ for calves on high concentrates, $24 \pm 2.4\%$ for calves on high hay, and $8 \pm 2.4\%$ for milk and reversal calves. These data agree with those of Emery (6) and Elliott (5), who found that rumen fill is predictable on the basis of the hay to concentrate ratio the animals are receiving. Corrections for body fill in calves on the basis of hay to concentrate ratio appear to be a potentially useful refinement for weights observed in growth trials. Studies which would form the basis of such corrections obviously need to be extended across a broader range of conditions than observed in the present experiment.

Proportion of rumen volume occupied by ingesta. As seen in Table 5, the rumens of the high-hay and high-concentrate calves 4 hr. post-prandial were approximately one-half full of solids and liquid, and the densities of the contents, as measured here, were about equal, viz., 1 kg. per liter. The calves which had consumed shavings, however, had filled their rumens to capacity with solid and liquid material, which also weighed 1 kg. per liter. The figure of one-half full may underestimate the space occupied by the contents in vivo, because the method used here to squeeze the liquid out of the rumen ingesta probably also squeezed out all entrapped gas. This gas probably contributes to the volume of the rumen contents in the live animal.

Stomach proportions. The relative proportions, as removed, of the total stomach of a hay-fed and milk-fed calf are shown in Figure 2. Most apparent is the reduced reticulo-rumen and omasal size and the greater size of the abomasum in the milk-fed tract. As seen in Figure 2, the hay-fed rumen is larger

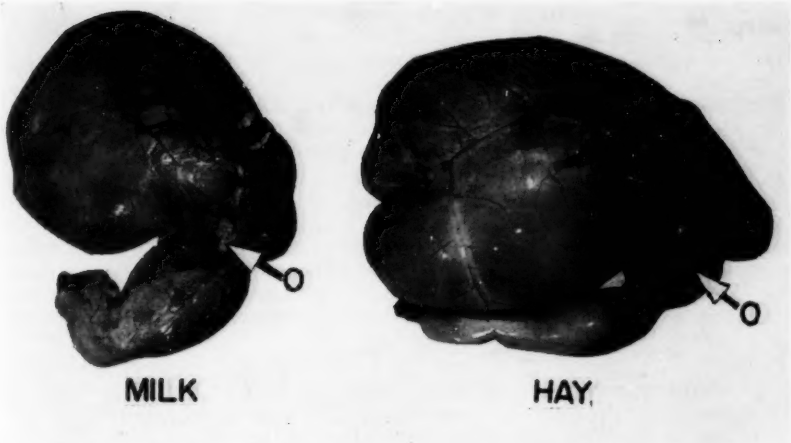

FIG. 2. Left: The digestive tract of a milk-fed calf photographed on approximately the same scale as the hay-fed calf on the right. The differences in the proportions occupied by the four stomach compartments is manifest. The omasum (located at the point of the arrows) in the milk-fed animal is practically undeveloped.

(55 vs. 18 liters). The milk-fed rumen can, however, expand to almost 50 liters when water-filled, whereas the hay rumen expands to 60 liters. Growth of the anterior and posterior blind sacs has occurred and appears to be similar to the post-natal rumen development of goats reported by Tamate (20).

Rumen development and growth. Length of papillae in the calves fed hay and grain was the only criterion in the present study which bore a positive relationship to the growth of the calves. Rumen volume, total tissue, and grams of tissue per dcm.^{2} all were greatest in the slower-growing high-hay calves or showed no difference between treatments. When one considers the moderately strong relationship between length of papillae and the growth of lambs shown by Sinclair and Kunkel (17), it is tempting to think of rumen papillae and rate of gain as having a cause-and-effect relationship. Such does not appear to be the case, however. Based on present knowledge of the forces causing papillary development, well-developed papillae and a rapidly growing animal are both the effects of a more basic cause, viz., an active rumen fermentation rich in sources of energy.

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UREA UTILIZATION BY YOUNG DAIRY CALVES AS AFFECTED BY CHLORTETRACYCLINE SUPPLEMENTATION[1]

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SUMMARY

Forty-eight two-day-old male and female calves representing the Holstein and Jersey breeds were raised to 86 days of age on a limited milk-hay-starter system to study the effect of chlortetracycline on urea utilization at three levels of protein equivalent intake. Four experimental starters ranging from 6.5 to 15.3% protein equivalent were fed with and without chlortetracycline supplementation. Highest average daily gains were made by calves on the 12.1% protein equivalent starter with chlortetracycline. The growth of calves increased as the protein equivalent level of the starter increased up to the 12.1% protein equivalent starter. Chlortetracycline did not spare dietary nitrogen in this study, but did significantly increase average daily gains, skeletal growth, and feed efficiency. Chlortetracycline had no significant effect on apparent digestibility of any ration nutrient, nitrogen retention, or blood levels of plasma protein and urea nitrogen. Coefficients of dry matter and crude protein digestibility, as well as nitrogen retention values, were significantly lower for calves on the 6.5% protein starter than for any of the other groups. The trend was for increased protein digestibility as the level of protein equivalent in the starter increased.

As reviewed by Lassiter (8), numerous workers have demonstrated the growth-promoting effects of chlortetracycline when fed to young dairy calves. Many hypotheses have been proposed for the mode of action of this antibiotic. For example, it has been suggested (3, 6, 12) that the mode of action in simple-stomach animals may be that of a protein-sparing effect. Everett *et al.* (4), working with calves, found no evidence of a protein-sparing effect in calves fed starters with protein levels ranging from 6.3 to 14.2% crude protein.

Limited data are available concerning the utilization of nonprotein nitrogen by young dairy calves. As early as 1942, Loosli and McCay (9) reported that 2-mo.-old calves receiving a high urea ration increased in body weight to 75-90% of normal at 4 mo. of age. In a later study, Knodt *et al.* (7), using ammoniated cane molasses and other similar products, observed that calves under 12 wk. of age were unable to utilize sufficient quantities of ammonia nitrogen to support normal growth. However, after 12 wk. the utilization of the ammoniated products increased markedly. In a previous report from this station (2), it was found that a portion of the nitrogen requirements of the young calf can be supplied in the form of urea when chlortetracycline is included in the ration.

The primary objectives of this study were to determine (a) the extent of urea utilization by young dairy calves and (b) to what degree urea utilization by the young calf is affected by chlortetracycline supplementation.

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EXPERIMENTAL PROCEDURE

Forty-eight two-day-old male and female calves representing the Holstein and Jersey breeds were allotted as they became available during the winter of 1956-1957 to eight groups, so as to equalize starting body weight with restrictions as to breed and sex. Each group, composed of three Jersey and three Holstein calves, was fed one of eight experimental starters (Table 1), with a

TABLE 1
Composition of calf starters

Ingredients	Starter			
	1 & 1A[a]	2 & 2A	3 & 3A	4 & 4A
	(lb.)			
Ground yellow corn	280	280	280	280
Ground oats	80	80	80	80
Corn starch	220	209	198	187
Molasses	100	100	100	100
Ground timothy hay	300	300	300	300
Urea	....	11	22	33
Salt	10	10	10	10
Steamed bonemeal	10	10	10	10
Vitamin A and D supplement (g.)[b]	500	500	500	500
	Chemical analysis (%)			
Dry matter	91.0	92.4	90.4	91.0
Crude protein	6.5	9.4	12.1	15.3
Ether extract	1.9	1.7	2.3	1.9
Crude fiber	11.2	12.2	11.7	11.0
N.F.E.	66.6	64.2	59.5	57.3
Ash	4.8	4.9	4.8	5.5
Nitrogen of ration supplied by urea	....	30.8	46.3	57.5

[a] A indicates that chlortetracycline was included in the starter.
[b] 10,000 I.U. vitamin A and 500 I.U. vitamin D per gram.

maximum daily intake limit of 6 lb. for Jersey and 8 lb. for Holstein calves. Chlortetracycline (Aurofac D) was added to the starters designated A at the rate of 15 mg. of chlortetracycline per pound of feed. Animals on the chlortetracycline-containing starters were fed 50 mg. of chlortetracycline daily in the milk up to 42 days of age. No hay was offered other than that mixed and pelleted in the starter. Since urea appeared to be utilized as early as 6 wk. of age in a previous study (2), the milk feeding schedule was altered in an effort to detect utilization of urea prior to this time. Based on actual weight each week, whole milk was fed at the daily rate of 8% of body weight for the first 21 days. From 22 to 42 days the level of milk feeding was reduced at weekly intervals to 6, 4, and 2% of body weight. Milk feeding ceased at 42 days of age.

All calves were weighed at two days of age and at weekly intervals thereafter throughout the 12-wk. experimental period. Skeletal measurements were made at two and 86 days of age. Daily records were kept of milk and starter consumption.

Digestion and nitrogen balance studies were conducted, using two male calves (one Jersey and one Holstein) from each experimental group at 5, 8, and 11 wk. of age. Chemical analyses of feed, feces, and urine were made by

A.O.A.C. methods (1). Venous blood samples were collected from two calves (one Jersey and one Holstein) in each group at two days and at 4, 8, and 12 wk. of age. Blood levels of plasma protein were determined by the micro-Kjeldahl method, and urea and nonprotein nitrogen were determined by the aeration method (5). The data obtained in this study were statistically analyzed by the analysis of variance (11). Covariance analysis was used in one specific instance. Where significant differences were indicated, the Hartley's multiple range test (11) was used to determine where these differences existed.

RESULTS

Growth. Satisfactory growth of all calves during the first 3 wk. indicated that the amount of whole milk being fed was sufficient to maintain normal growth regardless of protein level of the starter fed (Figure 1). The apparent need for additional nutrients after the 3rd wk. is demonstrated by reduced

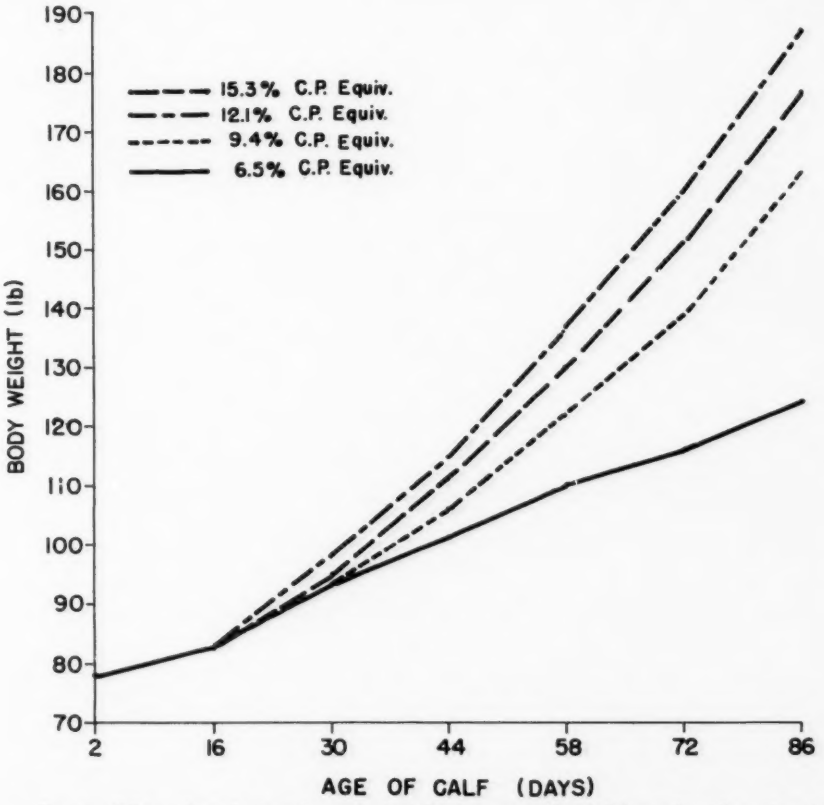

FIG. 1. Effect of crude protein equivalent level on growth of dairy calves from two to 86 days of age.

TABLE 2

Effect of protein equivalent level and chlortetracycline on growth of calves from two to 86 days of age

Starters	Av. initial wt.	Av. daily gain	Av. increase in Ht. at withers	Av. increase in Heart girth
	(lb.)		(in.)	
1	80	0.56	2.8	4.8
1A	80	0.51	2.4	5.2
Av. for 6.5% C.P.	80	0.54	2.6	5.0
2	78	0.84	3.9	7.5
2A	76	1.21	4.9	9.7
Av. for 9.4% C.P. equiv.	77	1.02	4.4	8.6
3	77	1.22	4.8	9.2
3A	80	1.36	4.9	9.9
Av. for 12.1% C.P. equiv.	78	1.29	4.9	9.5
4	76	1.08	4.5	8.7
4A	78	1.30	5.1	9.7
Av. for 15.3% C.P. equiv.	77	1.19	4.8	9.2
No chlortetracycline	78	0.92	4.0	7.5
Chlortetracycline	78	1.10[a]	4.3	8.6[a]

[a] ($P < 0.01$).

Significant differences:

Av. daily gain: Protein equivalent level: 2, 3, 4 > 1 ($P < 0.01$); 3 > 2 ($P < 0.01$) and 4 > 2 ($P < 0.05$). Ht. at withers: Protein equivalent level: 2, 3, 4 > 1 ($P < 0.01$). Heart girth: Protein equivalent level: 2, 3, 4 > 1 ($P < 0.01$).

growth of the 6.5% protein group and to a lesser extent by the 9.4% protein equivalent group (Figure 1). Since a difference in growth rate was observed at this time, it appears that calves can utilize urea as early as 3 wk. of age.

The average daily gains and increase in heart girth and height at withers are shown by starter groups in Table 2. All of these growth measurements followed the same trend by increasing as the protein equivalent level of the starter increased up to the 12.1% protein group. The slight differences between the 12.1 and 15.3% protein groups in these measurements partially may have been due to differences in feed consumption; however, both groups grew at normal rates (10). The three high groups by protein levels increased significantly more ($P < 0.01$) in body weight, height at withers, and heart girth than the low protein group (6.5% C.P.). No significant differences were observed among the 12.1 or 15.3% protein groups; however, both gained significantly more in body weight than the 9.4% protein group.

Chlortetracycline significantly ($P < 0.01$) increased body weight gains and heart girth. With the exception of gains exhibited by Group 1A, chlortetracycline gave average daily gains similar to the next higher protein level without chlortetracycline. This observation is in agreement with a previous report from this station concerning minimum protein levels (4). The average daily gains exhibited by the 6.5 and 15.3% protein groups without chlortetracycline compare closely with average daily gains of calves on comparable protein and protein equivalent levels reported in a previous study (2, 4).

Starter consumption and feed efficiency. Starter consumption (Table 3) increased with increasing levels of protein equivalent up to the 12.1% level. All groups by protein levels consumed significantly ($P < 0.01$) more starter

TABLE 3

Effect of protein equivalent level and chlortetracycline supplementation on feed consumption and efficiency of utilization

Starter	Average total consumption per calf		Feed/gain
	Milk	Starter	
	(lb.)	*(lb.)*	*(lb/lb)*
1	192.5	158.0	3.90
1A	200.4	137.2	3.83
Av. for 6.5% C.P.	196.4	147.6	3.86
2	208.7	202.8	3.30
2A	198.2	237.2	2.61
Av. for 9.4% C.P. equiv.	203.4	220.0	2.96
3	196.7	264.5	2.87
3A	214.6	290.1	2.86
Av. for 12.1% C.P. equiv.	205.6	277.3	2.86
4	195.4	254.2	3.11
4A	194.8	279.4	2.80
Av. for 15.3% C.P. equiv.	195.1	266.8	2.96
No chlortetracycline	198.3	219.9	3.30
Chlortetracycline	202.0	236.0[a]	3.02[a]

[a] ($P < 0.01$).

Significant differences: Starter consumption: Protein equivalent level: 2, 3, $4 > 1$ ($P < 0.01$); 3, $4 > 2$ ($P < 0.01$) and ($P < 0.05$). Feed/gain: Protein equivalent level: 2, 3, $4 > 1$ ($P < 0.01$).

than the 6.5% protein group. It was also found that the 12.1 and 15.3% protein groups consumed significantly ($P < 0.01$ and $P < 0.05$, respectively) more starter than the 9.4% group. Even though chlortetracycline stimulated starter consumption at every protein level, with the exception of the 6.5% protein group, the differences were not statistically significant. This trend, however, closely approximates that found in average daily gains. Chlortetracycline significantly ($P < 0.01$) increased gross efficiency of feed utilization. All groups by protein equivalent levels exhibited significantly higher ($P < 0.01$) gross feed efficiency values than the low protein group (6.5% C.P.).

Since gross feed efficiency does not give the efficiency of utilization of feed intake above maintenance, the total TDN intake and the TDN intake above maintenance was calculated. This was done by assuming that the maintenance requirement of young calves is 0.8 lb. TDN/100 lb. body weight per day. From these values the TDN above maintenance required per pound of gain was calculated and analyzed statistically. The mean of 1.49 lb. TDN above maintenance required per pound of gain for the calves that did not receive chlortetracycline was significantly greater ($P < 0.05$) than the comparable mean of 1.35 for the calves that received chlortetracycline.

There was also a significantly lower TDN intake above maintenance per pound of gain for the Holstein breed. However, the maintenance requirement assumed was a linear function of weight and the breed difference noted may be largely associated with a difference in body weight, the lower-weight animals actually having a higher maintenance requirement per unit weight. The TDN intake above maintenance per pound gain was significantly greater for Group 1 than any other group.

TABLE 4

Average coefficients of apparent digestibility and daily nitrogen retention

Starter	Dry matter	Crude protein	Crude fiber	Nitrogen retention	Per cent of N intake retained
	(%)			(g/day)	
1	69.3	52.6	28.9	2.8	19.7
1A	66.4	46.2	31.1	2.5	20.3
Av. for 6.5% C.P.	67.8	49.4	30.0	2.6	20.0
2	76.6	64.4	44.5	8.7	39.5
2A	73.6	65.4	31.1	8.7	34.0
Av. for 9.4% C.P.	75.1	64.9	37.8	8.7	36.8
3	74.7	72.0	34.8	10.9	34.5
3A	71.6	71.5	28.2	9.6	29.6
Av. for 12.1% C.P.	73.2	71.8	31.5	10.2	32.0
4	74.3	75.2	27.4	13.0	34.8
4A	72.4	73.8	25.3	12.1	30.6
Av. for 15.3% C.P.	73.4	74.5	26.4	12.6	32.7
No chlortetracycline	73.7	66.0	33.9	8.8	32.1
Chlortetracycline	71.0	64.2	28.9	8.2	28.6

Significant differences: Dry matter digestibility: Protein equivalent level: 2, 3, 4 > 1 ($P < 0.05$). Crude protein digestibility: Protein equivalent level: 2, 3, 4 > 1 ($P < 0.01$). Nitrogen retention: Protein equivalent level: 4 > 1 ($P < 0.01$); 3, 2 > 1 ($P < 0.05$).

Digestion coefficients and nitrogen balance. Digestibility and nitrogen balance data are given in Table 4. Coefficients of apparent digestibility of dry matter and crude protein were significantly lower for the lowest protein group than for the three other groups. In general, the trend was for increased protein digestibility as the level of protein in the starters increased. The differences in crude fiber digestibility among protein equivalent groups were not significant. There were no significant differences in nitrogen retention among the three highest protein levels, although the trend was for increased nitrogen retention with increasing protein levels in the starter. Chlortetracycline had

TABLE 5

Average plasma protein and blood urea levels for two calves per group

Ration	Week	Plasma protein				Blood urea nitrogen			
		0	4	8	12	0	4	8	12
		(g. %)				(mg. %)			
1		5.9	5.5	5.7	5.6	12	4	1	6
1A		5.9	5.3	5.2	5.1	4	3	2	3
Av. for 6.5% C.P.		5.9	5.4	5.4	5.4	8	4	2	4
2		6.0	5.5	5.6	5.6	8	5	3	5
2A		5.8	5.5	5.2	5.2	11	3	2	3
Av. for 9.4% C.P. equiv.		5.9	5.5	5.4	5.4	10	4	2	4
3		6.7	5.5	5.2	5.2	15	7	7	5
3A		5.7	5.3	5.3	5.8	19	8	8	7
Av. for 12.1% C.P. equiv.		6.2	5.4	5.2	5.5	17	8	8	6
4		5.7	5.0	5.2	5.3	14	9	8	6
4A		5.8	5.4	5.6	5.3	18	10	21	6
Av. for 15.3% C.P. equiv.		5.8	5.2	5.4	5.3	16	10	14	6
No chlortetracycline		6.1	5.4	5.4	5.4	12	6	5	6
Chlortetracycline		5.8	5.4	5.3	5.4	13	6	8	4

Significant differences: Plasma protein: Period: Week 0 > weeks 4, 8, and 12 ($P < 0.01$). Blood urea nitrogen: Period: Week 0 > weeks 4, 8, and 12 ($P < 0.01$).

no significant effect on the digestibility of any nutrient fraction or on nitrogen retention.

Plasma protein and blood urea levels. As may be noted in Table 5, chlortetracycline had no significant effect on blood levels of plasma protein or urea nitrogen. Protein levels in the starter did not significantly affect blood plasma protein levels. However, it was observed that calves on the 15.3% protein equivalent starter had higher levels of blood urea nitrogen than calves on either the 6.5 or 9.4% protein equivalent starters. Significant period differences were observed with all groups exhibiting significantly higher ($P < 0.01$) levels of both plasma protein and blood urea nitrogen at two days of age than at 4, 8, or 12 wk. of age.

DISCUSSION

One criterion for determining the nutritional adequacy of any feedstuff for young animals is growth. Calves which received the 6.5% protein starter grew at subnormal rates, which apparently could be attributed to the low protein content of the starter. Since all of the urea-supplemented groups exhibited somewhat greater average daily gains than the 6.5% protein group, there is little doubt as to the ability of the young calf to utilize urea as a dietary source of nitrogen. Since a limited amount of milk was fed for the first 6 wk. of the calf's life, the earliest age at which the rumina of calves are developed to the extent of utilizing urea was not determined. Based on this study, as well as on a previous study (2), it appears that little difference in growth occurred when the calves received as much whole milk as 4 to 6% of their body weight per day. When comparing weekly gains of all calves, by protein and protein equivalent levels, it appears that at least a portion of the urea was being utilized as early as 3 wk., since a growth advantage was noted in the urea-supplemented groups at that time.

Since both starter consumption and body weight gains increased as the protein level of the starter increased, it might appear that the differences in body weight could be accounted for totally by starter consumption. This does not appear to be the only factor, however, as shown in Table 6. When body weight gains among protein equivalent levels were adjusted for differences in starter consumption, the differences in weight gain remained highly significant, even though the regression of body weight gain on starter consumption was significant ($P < 0.01$). This indicates that even though a considerable portion

TABLE 6

Analysis of covariance[a]

Source	dF	X M.S.	XY M.P.	Y M.S.	Adj. M.S.
Breeds	1	61,812	29,911	14,560	
C. P. equiv.	3	41,963	19,740	9,459	355[b]
Chlortetracycline	1	3,485	2,790	2,494	1,126[b]
Error	42	1,999	733	320	50

[a] X = starter consumption. Y = body weight gains.
[b] Significant ($P < 0.01$).

of the variation in growth can be explained on the basis of starter consumption there remains a significant portion that must be associated with the use of chlortetracycline and dietary nitrogen intake.

Chlortetracycline significantly increased average daily gains, but failed to affect starter consumption significantly. There was a definite trend, however, for greater starter consumption by the calves receiving chlortetracycline. When corrected for starter consumption (Table 6), the growth-promoting effect of chlortetracycline was even more pronounced, which indicates that, in this study at least, the mode of action of chlortetracycline was not solely through appetite stimulation.

Using the criteria proposed by Jensen *et al.* (6), chlortetracycline failed to spare dietary protein in this study, since it did not (a) significantly improve nitrogen retention, (b) give a consistently greater growth response at lower protein levels than at higher protein levels, and (c) give a significant protein × chlortetracycline interaction.

Even though chlortetracycline did not spare dietary protein in this study, it was observed that chlortetracycline-fed calves made satisfactory gains on starter containing approximately 3% less protein equivalent than that required to produce similar gains when chlortetracycline was not fed.

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SURVIVAL OF BOVINE SPERMATOZOA STORED AT 5 AND 25° C. IN EXTENDERS CONTAINING VARYING LEVELS OF EGG YOLK, GLUCOSE, GLYCINE, GLYCEROL, CITRATE, AND OTHER SALTS

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SUMMARY

Combinations of 20% egg yolk with 1.5 to 3.0% solutions of glycine resulted in poor survival of spermatozoa. Inclusion of 10% glycerol resulted in sperm survival comparable to that obtained in 50% egg yolk–glycine extenders with or without glycerol. A 20% egg yolk extender containing 1.16% sodium citrate dihydrate, 0.75% glycine, and 1.0% glucose was evolved. Twenty ejaculates of semen stored in this extender averaged 41% motile spermatozoa after 12 days of storage at 5° C. Results at 5° C. were superior to those at 25° C. ($P < 0.01$), even when 0.3% sulfanilamide was added. A yolk–citrate–glucose–glycine–sulfanilamide–antibiotic extender, designated as CU-16, was compared with one designated as CUE, which contained in addition sodium bicarbonate, potassium chloride, and citric acid. In two experiments the percentage of motile spermatozoa over a 12-day storage period at 5° C. in CU-16 averaged 49 and 52, and in CUE averaged 50 and 52 ($P > 0.05$). The high sperm survival in both of these extenders at 5° C. indicated they might be useful in preserving the fertility of sperm stored for several days at this temperature.

The maximum utilization of outstanding sires in artificial breeding depends in part upon the frequency with which they are ejaculated, and in part upon the preservation of maximum fertility of the spermatozoa between semen collections. Since bulls can be ejaculated routinely at least twice a week with no effect on fertility (4, 12), extenders preserving high fertility of unfrozen semen for three to four days would help increase the over-all conception rate, encourage more complete use of semen, and make semen from most of the outstanding sires continuously available in the field. While considerable progress has been made in defining the environmental conditions favorable for sperm survival, the fact that simple changes in the medium or storage conditions often result in longer survival than was previously possible indicates that optimal conditions have not been determined.

The yolk-phosphate extender developed by Phillips (14) and the yolk-citrate extender developed and modified by Salisbury *et al.* (16, 17) maintain good fertility of the semen for only about two days. Semen extended with milk or milk-glycerol (2) has been used over a four-day period, but there is a considerable decrease in nonreturn rate during this time (2, 3). Foote (9) found that complex buffer solutions were helpful in prolonging survival of spermatozoa, and Tyler and Tanabe (18) and Roy and Bishop (15) found glycine to be beneficial. Foote and Bratton (10) reported that antibacterial agents improved sperm survival at room temperature, and VanDemark and Sharma (19) prolonged survival of spermatozoa at this temperature with carbon dioxide. However, none of the methods reported have consistently maintained high motility when the semen was stored over a period of several

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days. The objective of the studies reported here was to determine those combinations of ingredients and storage conditions at 5 and 25° C. that would increase the survival time of bovine spermatozoa.

EXPERIMENTAL PROCEDURE

Buffers were prepared aseptically, using boiling redistilled water. After combining egg yolk and buffer, 1,000 units of penicillin and 1,000 μg. of dihydrostreptomycin were added per milliliter of extender, with the exception noted in Table 3. Semen was added to the extenders at a rate to give ten million motile spermatozoa per milliliter of extender. Samples stored at 5° C. were cooled to this temperature from 30° C. over a period of 75 min. Storage was either in 3-ml. test tubes containing 2 ml. of extended semen, or in 1.5-ml. glass pull-seal ampules containing 1 ml. of extended semen. After various intervals of storage the semen in the test tubes was mixed, subsampled, and the percentage of motile spermatozoa estimated microscopically at 430 magnifications in a stage incubator at 37° C. A different set of ampules was mixed and opened for examination at each corresponding interval of storage.

The pH's of the extenders and extended semen were determined routinely with a Beckman Model G pH meter. The analyses of variance were computed as described by Henderson (13). Bulls were considered to be a random variable, whereas all other variables, extenders, days stored, etc. were considered to be fixed effects. Duncan's Multiple Range Test (5) was used to test the significance of the differences between ranked means.

RESULTS AND DISCUSSION

Yolk-glycine-glycerol extenders at 5° C. Twenty per cent egg yolk by volume was added to aqueous solutions of glycine and glycerol, and compared with 20% egg yolk–citrate controls. The concentrations of the ingredients (weight in volume) in the final extenders and the percentage of motile spermatozoa during storage in these extenders are shown in Table 1. The livability of the spermatozoa in the yolk-glycine extenders was poor. A highly significant improvement ($P < 0.01$) in sperm survival resulted when 10% glycerol was added. The mechanism by which glycerol produced this dramatic effect is not known. The pH did not appear to be a factor, as the pH of all extenders was similar.

TABLE 1

Percentage of motile spermatozoa in 20% yolk-glycine-glycerol extenders stored at 5° C. (Mean of 13 ejaculates)

Days stored at 5° C.	2.32% Sodium citrate control		0% Glycerol			10% Glycerol		
	0% Glycerol	7% Glycerol	1.5% Glycine	2.25% Glycine	3.0% Glycine	1.5% Glycine	2.25% Glycine	3.0% Glycine
	(percentage of motile spermatozoa)							
1	54	55	14	28	32	48	49	50
2	45	49	3	9	15	35	44	45
3	41	33	5	7	15	27	35	36
Mean	47	46	8	15	21	37	43	44

Since Roy and Bishop (15) had reported favorable results using a 50% yolk-glycine extender the same series of treatments shown in Table 1, plus a similar series with 50% egg yolk were compared over a ten-day storage period, using eight ejaculates of semen. The percentages of motile spermatozoa in 50% egg yolk–glycine with and without glycerol averaged 33 and 37, respectively, and in 20% egg yolk–glycine with and without glycerol, 31 and 9, respectively. The strikingly beneficial effect of glycerol in the 20% egg yolk–glycine series confirmed the results shown in Table 1, and is an effect not previously reported in the literature, so far as the authors have been able to find. The minor effect of glycerol in the 50% egg yolk–glycine series agrees with the reports by Albright *et al.* (1) and Flipse and Almquist (7). The interaction between levels of egg yolk and glycerol was found to be highly significant statistically ($P < 0.01$).

In another series of experiments, semen was stored over an eight-day period at 5° C. in 20 and 50% yolk extenders containing phosphate and citrate buffers (14, 17). mixed in equal proportions with the previously tested glycine-glycerol buffer combinations. The substitutions with citrate and phosphate buffers reduced the final glycine and glycerol concentrations to one-half those shown in Table 1. The major effects and statistical significance of the differences in these experiments over the eight-day period were as follows:

1. 20% yolk extenders were superior to 50% yolk extenders, averaging 40% vs. 34% motile spermatozoa, respectively ($P < 0.01$).
2. Citrate buffer was superior to phosphate buffer, averaging 42 vs. 32% motile spermatozoa, respectively ($P < 0.01$).
3. Yolk-glycine-citrate and yolk-glycine-phosphate extenders containing 0 and 5% glycerol did not differ, averaging 39 vs. 38% motile spermatozoa, respectively ($P > 0.05$).
4. Inclusion of 0.75, 1.125, and 1.50% glycine resulted in an improvement in sperm survival compared to no glycine, averaging 44, 41, and 40% vs. 25% motile spermatozoa, respectively ($P < 0.01$).

The extender having the highest average percentage of motile spermatozoa (52%) over the eight-day storage period was 20% yolk containing 1.16% sodium citrate dihydrate and 0.75% glycine. It was superior to the control 20% yolk extender containing a final concentration of 2.32% sodium citrate dihydrate, and was selected for further testing. Because the 1.16% sodium citrate dihydrate included in the experimental extender (1.45% in the buffer) was only one-half the amount found by Salisbury *et al.* (17) to be isotonic with bull semen, and because the inclusion of 0.75% glycine was not considered to fully compensate osmotically for the reduction in the citrate component, an increase in the concentration of solutes in the buffer seemed warranted. Increases in the level of sodium citrate and the addition of dextrose were studied as a means of further improving this extender. The concentrations of the various ingredients in the extenders and the percentage of motile spermatozoa during storage are shown in Table 2. The analysis of variance indicated that the treatments differed significantly ($P < 0.01$), with the percentage

TABLE 2

Percentage of motile spermatozoa in 20% yolk-citrate-glycine-glucose extenders stored at 5° C. (Mean of 20 ejaculates)

Days stored at 5° C.	0.75% Glycine						Control 2.32% citrate
	1.16% Citrate		1.45% Citrate		2.00% Citrate		
	0% Glucose	1.00% Glucose	0% Glucose	1.00% Glucose	0% Glucose	1.00% Glucose	
	(percentage of motile spermatozoa)						
1	64	64	63	63	62	64	60
2	62	64	61	62	56	58	56
4	58	60	56	54	52	52	50
8	52	54	50	46	47	48	43
12	38	41	35	34	35	36	26
Mean	55	56	53	52	50	51	47

of motile spermatozoa tending to decrease as the level of sodium citrate increased. Inclusion of glucose resulted in a small but nonsignificant over-all increase in sperm survival. The motility of the spermatozoa in the extender containing 0.75% glycine, 1.16% sodium citrate, and 1.00% glucose declined only four percentage units during the first four days of storage.

Simple versus complex extenders at 5 and 25° C. In previous studies by Foote (8, 9) it had been observed that complex buffers, such as the experimentally modified Tyrode's shown below, combined with whole egg (6) preserved the motility of spermatozoa at a high level for 48 hr. at 25° C.

Ingredients	Grams per liter
NaCl	8.0
KCl	0.2
$CaCl_2$	0.2
$MgCl_2$	0.1
Glucose	1.0
$NaHCO_3$	0.5
NaH_2PO_4	1.0
Distilled water to 1,000 ml.	
Citric Acid to adjust pH to 7.0	

The percentages of motile spermatozoa during 48 hr. storage at 25° C., when this buffer was combined with whole egg, whole egg–glucose, and egg yolk, are shown in Table 3. The best results were obtained with Tyrode's–whole egg or whole egg–glucose combinations, Treatments 2 and 3. Other studies by Foote (9) showed that neither Ca^{++} nor Mg^{++} improved sperm survival, but that sulfanilamide was beneficial at storage temperatures of 25° C. (8, 10).

It was difficult to observe the spermatozoa microscopically in the egg yolk extender, No. 4, of Table 3. This difficulty was obviated by replacing the sodium chloride with sodium citrate and glycine. Also, the levels of sodium bicarbonate, potassium chloride, and glucose were adjusted, for comparative purposes, to those reported by VanDemark and Sharma (19). The compositions of three extenders evolved from this study and those given in Table 2 are shown in

TABLE 3

Percentage of motile spermatozoa in modified Tyrode's–whole egg and egg yolk extenders stored at 25° C.
(Mean of ten ejaculates)

Hours stored at 25° C.	No. 1 Tyrode's only	No. 2 4 Parts No. 1 to 1 part whole egg	No. 3 4 Parts No. 2 to 1 part 5% glucose	No. 4 4 Parts No. 1 to 1 part egg yolk	No. 5[a] 2.9% Citrate whole egg
		(percentage of motile spermatozoa)			
8	59	63	65	64	65
24	50	63	61	60	61
48	32	55	56	50	48
Mean	47	60	61	58	58

[a] 2.9% Sodium citrate–sulfasuxidine–whole egg (6) contained 500 units of penicillin and 500 μg. of streptomycin per milliliter, as did Treatments 1-4.

Table 4. Two of these extenders are referred to in a preliminary report by Foote *et al.* (11) as CU-16 and CUE. The results of the motility studies and pH determinations with these extenders are shown in Table 5. The analysis of variance revealed that at 25° C. extenders differed, and application of Duncan's Multiple Range Test (5) to the ranked treatment means showed that the inclusion of sulfanilamide (No. 1 vs. No. 2) was beneficial ($P < 0.05$). The percentage of motile spermatozoa at 5° C. was higher than at 25° C. ($P < 0.01$). The decrease in pH during storage was much more marked at 25 than at 5° C., especially in the absence of sulfanilamide.

TABLE 4

Composition of buffers and extenders used for preserving spermatozoa at 5 and 25° C.

Ingredients	Composition No. 1	No. 2 (CU-16)	No. 3 (CUE)
Buffers:			
Sodium citrate dihydrate *(g.)*	14.5	14.5	14.5
Sodium bicarbonate *(g.)*	.....	.....	2.1
Potassium chloride *(g.)*	.....	.....	0.4
Glucose *(g.)*	12.5	12.5	3.0
Sulfanilamide *(g.)*	.....	3.0	3.0
Glycine *(g.)*	9.37	9.37	9.37
Citric acid *(g.)*	.....	.....	0.87
Distilled water (final volume) *(ml.)*	1,000	1,000	1,000
Extenders:[a]			
Buffer *(% by volume)*	80	80	80
Egg yolk *(% by volume)*	20	20	20
Penicillin *(units/ml)*	1,000	1,000	1,000
Dihydrostreptomycin *(μg/ml)*	1,000	1,000	1,000

[a] The concentration of the buffer ingredients in the extender is 80% of those in the buffer, due to the addition of 20% egg yolk to prepare the extender.

Sealed ampules vs. corked tubes. The motility of spermatozoa stored in CU-16 and CUE at 5 and 25° C. in sealed glass ampules versus corked glass tubes is summarized in Table 6. The analysis of variance revealed that storage interval and storage temperature had marked effects on sperm survival ($P < 0.01$). The difference between extenders ($P < 0.05$) was due to the difference in survival rate of the semen in CU-16 stored in ampules, whereas both ex-

TABLE 5

Percentage of motile spermatozoa and pH of extended semen stored at 5 and 25° C. (Mean of ten ejaculates)

Days stored	Stored at 5° C.			Stored at 25° C.		
	No. 1	No. 2 (CU-16)	No. 3 (CUE)	No. 1	No. 2 (CU-16)	No. 3 (CUE)
	(percentage of motile spermatozoa)					
1	61	59	63	59	62	60
4	61	61	62	47	57	52
8	43	48	45	30	32	24
12	22	27	30	18	23	22
Mean	47	49	50	38	44	40
	(pH's)					
0	6.86	6.85	6.92	6.86	6.85	6.92
12	6.59	6.72	6.87	6.03	6.39	6.67

tenders gave similar results when stored in test tubes. This difference is reflected in the statistically significant extender × container interaction. None of the other interactions was statistically significant.

The decrease in pH during storage was especially pronounced at 25° C. in corked tubes. The high pH's after storage in CUE in ampules at 5° C. may have been partly due to evolution of CO_2 between the time the ampules were opened for estimating the motility of the spermatozoa and the time the pH's were determined. In addition, some CO_2 may have evolved prior to sealing the ampules, resulting in a high pH during storage and in a lower motility than normal in the CUE extender. Occasionally, unexplained rises in the pH of CUE to slightly above the neutral point occurred, although the inclusion of the citric acid prevented major shifts in pH which accompany loss of CO_2 in highly carbonated buffers.

To test the efficacy of CU-16 and CUE in preserving the spermatozoa obtained from a larger segment of bulls, 30 additional samples of semen were stored in these extenders in test tubes at 5° C. Over a 12-day storage period

TABLE 6

Percentage of motile spermatozoa and pH of extended semen stored in CU-16 and CUE at 5 and 25° C. in sealed ampules and corked tubes (Mean of ten ejaculates)

Days	Stored at 5° C.				Stored at 25° C.			
	Ampules		Corked tubes		Ampules		Corked tubes	
	CU-16	CUE	CU-16	CUE	CU-16	CUE	CU-16	CUE
	(percentage of motile spermatozoa)							
1	66	63	61	63	61	55	62	58
4	62	56	61	60	53	44	54	47
8	49	38	51	47	36	24	35	32
12	42	33	36	37	22	22	16	24
Mean	55	48	52	52	43	36	42	40
	(pH's)							
0	6.98	6.99	6.98	6.99	6.98	6.99	6.98	6.99
12	6.87	7.28	6.77	7.05	6.48	6.76	6.18	6.48

the percentages of motile sperm in the CU-16 and in the CUE averaged 50 and 52, respectively ($P > 0.05$). These percentages are considerably higher than has generally been reported for bovine sperm stored for 12 days at 5° C., despite the fact that in all of the trials reported here the semen was extended on the average more than 100 times. The results of fertility trials with these extenders are being reported.

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FERTILITY OF BULL SEMEN STORED UP TO FOUR DAYS AT 5° C. IN 20% EGG YOLK EXTENDERS

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SUMMARY

Three fertility trials comparing experimental extenders CUE and CU-16 with a standard 50% egg yolk–citrate–sulfanilamide control were conducted in 1957 and 1958. Semen was extended to ten million motile spermatozoa per milliliter of extender in all trials. The first trial included four bulls, and the semen was used for insemination primarily one and two days after collection. A second field trial was designed to use semen extended with CUE and CU-16 more extensively three to four days after collection, and a third field trial, conducted on a larger scale, was designed to obtain results with all three extenders over a four-day storage period. The combined number of first services and weighted mean percentages of 60- to 90-day nonreturns for all three trials was 6,069 and 73.4 for the control 2.9 CSAY, 6,280 and 76.6 for the CUE, and 6,145 and 76.0 for the CU-16. The adoption of CUE for routine use by the local artificial breeding cooperative resulted in an immediate and consistent increase of about two percentage units, bringing the 60- to 90-day nonreturn rate to an average of 74.9% for nearly 300,000 first inseminations.

Since the development of egg yolk–phosphate and egg yolk–citrate extenders (8, 9), many buffered-yolk extenders have been formulated. A recent publication by Foote and Bratton (4) described a series of experiments leading to the development of the CUE and CU-16 extenders for bovine semen. These extenders preserved spermatozoa stored at 5° C. in corked tubes at a higher level of motility than has generally been reported in the literature for either egg yolk or milk extenders. This suggested that they might preserve the fertility of the spermatozoa stored under similar conditions more effectively than other extenders in common use. This paper reports the fertility results of three field trials in which the improved extenders, CUE and CU-16, were compared with a standard 50% egg yolk–citrate control. No published extensive fertility tests with extenders of these types were found in the literature, although during the course of these studies reports have appeared on the use of milk or 50% egg yolk combined with glycine, and a number of other ingredients (1, 7, 10, 11).

EXPERIMENTAL PROCEDURE

The composition of the buffers and extenders, along with the pH of the extenders, used in the field trials is shown in Table 1. The CUE buffer was prepared by weighing all of the dry ingredients excepting citric acid into a 1-liter sterile Erlenmeyer flask. Approximately 800 ml. of water redistilled in glass was added, and the mixture stirred rapidly while it was heated to a boil.

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TABLE 1
Composition and pH of buffers and extenders

Ingredients	2.9 CSAY (control)	CUE	CU-16
Buffers:			
Sodium citrate dihydrate *(g.)*	29.0	14.5	14.5
Sodium bicarbonate *(g.)*	.....	2.1	.....
Potassium chloride *(g.)*	.....	0.4	.....
Glucose *(g.)*	.....	3.0	12.5
Glycine *(g.)*	.....	9.37	9.37
Sulfanilamide *(g.)*	6.0	3.0	3.0
Citric acid *(g.)*	.....	0.87	.....
Distilled water (final volume) *(ml.)*	1,000	1,000	1,000
Extenders:			
Buffer *(% by volume)*	50	80	80
Egg yolk *(% by volume)*	50	20	20
Penicillin *(units/ml)*	500	1,000	1,000
Dihydrostreptomycin *(μg/ml)*	500	1,000	1,000
pH's of extenders:			
Trial 1	6.75	6.82	6.86
Trial 2	6.66	6.88	6.77
Trial 3	6.71	6.91	6.80

Even with rapid stirring, glycine had a tendency to adhere to the glass and decompose when direct heat was applied. Any batches in which this occurred were discarded. Subsequently, it was found that the buffer could be simply prepared, with apparently satisfactory control of bacteria, merely by dissolving the dry ingredients in boiling redistilled water. The latter procedure was followed in Field Trial 3. The hot buffer was either cooled overnight in the dark, or rapidly under a stream of cold water. Fifteen milliliters of a previously prepared 5.8% stock solution of citric acid was added to adjust the pH. The buffer was transferred to a 1-liter volumetric flask and brought to final volume by adding cool sterilized redistilled water. The buffer was stored at room temperature in the dark.

The 2.9 CSAY and CU-16 buffers were prepared in a similar manner, but without the addition of citric acid. The extenders were prepared fresh daily by combining egg yolk with the buffers, and adding antibiotics to the final extenders in the amounts shown in Table 1.

Semen was collected at weekly intervals from bulls in the stud of the New York Artificial Breeders' Cooperative, Inc. It was processed as described by Foote and Bratton (3), and finally extended to give ten million motile spermatozoa per milliliter (2). The average ratios of semen to extender to accomplish this in Trials 1, 2, and 3 were 1:105, 1:113, and 1:114, respectively. The extended semen was dispensed into plastic tubes for shipment to the inseminating technicians. To maximize the number of breedings possible in each experimental subunit, each semen sample normally was shipped to all technicians. The treatments were randomized to bulls and to days of the week. This resulted in all treatments being tested in the same week, thus avoiding any confounding of treatment with time trends. Over a 3-wk. period the semen from each bull was extended with all three extenders; thus, there was no confounding of bulls with treatments. Fertility was measured by the percentage of first-service cows

not requiring reinsemination within 60 to 90 days following service. Analyses of variance of these data were made according to the procedures outlined by Henderson (6).

RESULTS AND DISCUSSION

Field Trial 1. This trial was conducted for 3 wk. in August and September, 1957, using the semen from four Holstein bulls. A preliminary account of this trial has been reported (5). The objective of this trial was to compare the fertility of the experimental extenders with the 50% yolk-citrate extender, under the standard procedures employed by the New York Artificial Breeders' Cooperative, Inc. In this study no effort was made to use the semen after longer periods of time than normal.

Field Trial 2. This trial was conducted in January and February, 1958, using the semen from one Guernsey, two Ayrshire, and two Jersey bulls. An effort was made to use the semen stored in the experimental extenders for more than two days, by holding a portion of each of the ejaculates extended with the two experimental extenders at the central bull stud for two days. At the end of this time extended semen was dispensed into 4-ml. plastic tubes and shipped to the technicians to be used three to four days after collection. This resulted in a higher proportion of the experimental semen being used after three to four days of storage than in the first trial.

Field Trial 3. This trial was conducted in June, 1958, using the semen from ten Holstein bulls. The proportion of breedings with three- to four-day-old semen was increased in all three extenders by storing a portion of the extended semen at the stud for 48 hr. in 2-ml. plastic tubes. At this time it was sent into the field for use three to four days after collection. The number of first inseminations and the percentage of cows not returning to service for all three trials are shown in Table 2.

The differences in the percentage of nonreturns among treatments were not statistically significant ($P > 0.05$) with the limited number of breedings obtained in the first two trials. However, despite the more extensive use of older semen stored in the experimental extenders in Trial 2 the average nonreturn rate was equal to or higher than the control. In Trial 3 the over-all difference of 3.9 percentage units between CUE and 2.9 CSAY control approached statistical significance, $P \cong 0.07$. The over-all results of the three trials suggest that a real improvement in nonreturn rate may be brought about by the use of CUE or CU-16 extenders. Since November 27, 1958, the New York Artificial Breeders' Cooperative, Inc. has used the CUE routinely.[2] The level of fertility increased simultaneously with this change, and the 290,053 first inseminations through May, 1959, using CUE-extended semen, have resulted in an average 60- to 90-day nonreturn rate of 74.9%. This level of fertility is two percentage units higher than for the comparable period in the previous year. Since no other known change in procedure was introduced, this sudden and consistent improvement in fertility tends to support the

[2] For routine use the citric acid content of the buffer was increased slightly, to 1.0 g. per liter.

TABLE 2

Number of first services and the 60- to 90-day percentage of nonreturns in three field trials

Field trial	Age of semen used for insemination[a]	2.9 CSAY		CUE		CU-16	
		No. of 1st services	% N.R.'s	No. of 1st services	% N.R.'s	No. of 1st services	% N.R.'s
I	0–1 day	631	78.6	614	79.6	684	76.6
	2 days	373	71.3	386	75.4	399	73.4
	3–4 days	18	72.2	23	56.5	46	76.1
	Total 1st services	1,022		1,023		1,129	
	Weighted % N.R.'s		75.8		77.5		75.5
II	0–1 day	319	76.2	393	78.1	319	82.8
	2 days	167	77.2	157	72.0	156	74.4
	3–4 days	20	30.0	205	70.7	156	69.9
	Total 1st services	506		755		631	
	Weighted % N.R.'s		74.7		74.8		77.5
III	0–1 day	2,968	74.2	3,061	77.6	2,944	77.1
	2 days	790	72.0	613	75.4	722	74.0
	3–4 days	783	67.4	828	73.9	719	72.6
	Total 1st services	4,541		4,502		4,385	
	Weighted % N.R.'s		72.7		76.6		75.9
Over-all three trials							
	Total 1st services	6,069		6,280		6,145	
	Weighted % N.R.'s		73.4		76.6		76.0

[a] Semen was collected prior to 6 A.M. The small amount used on Day 0 was 6 to 12 hr. old when used and proportionately older on subsequent days in all field trials.

controlled experimental results. For an organization breeding 500,000 cows this improvement represents 10,000 more cows conceiving to first service, or one more cow in each 50-cow dairy. Studies are being continued with the hope of further improving the fertility of extended semen stored at 5° C. for several days.

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FREQUENCY OF EJACULATION IN THE BOVINE[1]

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SUMMARY

Data from one set of monozygotic triplet Holstein bulls indicated that at least seven successive ejaculates could be collected once each week for at least a year with no detrimental effects on semen quality or libido. The bulls giving four and seven ejaculates per week produced 2.4 and 3.0 times as many spermatozoa as the bull giving two ejaculates per week.

Additional data from one set of monozygotic triplet and three sets of monozygotic twin Holstein bulls indicated that the collection of two ejaculates every two days or four days for a period of 2 yr. did not result in appreciable loss in libido or semen quality as compared to bulls giving two ejaculates every seven days. Bulls giving two ejaculates every two and four days produced 2.1 and 1.2 times as many spermatozoa as bulls giving two ejaculates once every seven days.

With the constantly increasing use of frozen semen the possibility of an increased number of ejaculates per collection period and the determination of the optimum time interval between collection periods became important.

Kirillov and Morozov (7) collected bull semen at the rates of one, two, and three ejaculates per day and found no ill effects on the bulls or the semen.

Baker *et al.* (1), working with nine Holstein bulls, collected semen at the rates of one, two, and three ejaculates per week. No significant differences were noted in semen characteristics, although a highly significant reduction of libido was noted in those bulls collected three times per week.

Hafs (6) collected semen from three bulls daily and reported lower volumes per ejaculate, spermatozoa concentration, and spermatozoa per ejaculate than three controls once a week. Daily collection did not measurably affect libido or body weight.

Bratton and Foote (2) and Bratton *et al.* (3) collected semen at the rate of one ejaculate every fourth and two ejaculates every eighth day. These collection schedules were not detrimental to either semen production or fertility for periods up to 1 yr.

Graham (5) found that increasing the collection frequency from two to four ejaculates per week decreased the volume of semen and spermatozoa per ejaculate but increased the volume and total spermatozoa per unit time.

At the rate of three collections per week VanDemark *et al.* (8, 9) collected a single bull for a 4-yr. period without detrimental effects on the semen quality or quantity.

Until this time little work had been done on frequency of ejaculation experiments for periods of over 1 yr. or on more than a limited number of animals. The present experiments were undertaken to study the effects of high frequencies

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of ejaculation on libido and semen characteristics over an extended period of time, utilizing monozygotic sets of twin and triplet bulls.

MATERIALS AND METHODS

A set of monozygotic triplet bulls was subjected to a preliminary uniformity trial of 4 mo. duration, which showed the bulls were uniform in all libido and semen characteristics studied. The bulls were then randomly assigned to a collection schedule of two, four, or seven successive ejaculates each seven days. They were maintained on these frequencies of collection for one year.

To test the effects of varying intervals of time between collection periods three sets of monozygotic twin Holstein bulls and one set of monozygotic triplet Holstein bulls were subjected to a 4-mo. uniformity period, after which the three sets of twin bulls were assigned to collection frequencies on an incomplete block, split plot design. Three collection frequencies were used, two ejaculates every two, four, or seven days. One bull of the set of triplets was assigned to each of the three frequencies. This study was conducted over a 2-yr. period and is being continued at the present time.

Collections were made with an artificial vagina in a special collecting room at one end of the bull barn. The bulls were exercised every other day for approximately 3 hr. Good-quality alfalfa hay was fed daily. No silage or supplemental pasture was provided. All animals were housed in the stall-type bull barn at the University of Minnesota.

Libido was measured by five characteristics: (a) preliminary time measured in minutes as the time from when the animal leaves his stall, until he passes seminal fluid from the sheath, plus 1 min., (b) reaction time measured in minutes as the time from release of the bull 1 min. after first passage of fluid from the sheath until actual ejaculation, (c) excitement score based on assigned values ranging from one to 14 as described by Frederick (4), one indicating a lack of excitation and 14 indicating an advanced state of excitement, (d) thrust score, measured on a scale of one to five as described by Frederick (4), one indicating a weak thrust and five indicating a very vigorous thrust, and (e) percentage of successful mounts.

Semen quality was measured by percentage of initial motility and of abnormal spermatozoa. Semen quantity was measured by volume per ejaculate, volume per unit time, spermatozoa per ejaculate, spermatozoa per unit time, and spermatozoa per milliliter.

RESULTS AND DISCUSSION

I. Effects of varying numbers of successive ejaculates per collection period.

Analysis of variance of the data collected showed a significant ($P = <0.05$) difference between treatments on preliminary and reaction times. A highly significant ($P = <0.01$) difference was found between treatments on thrust scores. In all cases the higher-frequency bull (seven ejaculates every seven days) showed a lower libido, as indicated by the characteristics studied, than did his pair-mates (Table 1).

TABLE 1

Mean values for libido and semen characteristics for varying numbers of successive ejaculates per collection period

Characteristic	Frequency of ejaculation		
	2 per week	4 per week	7 per week
Preliminary time[a] *(min.)*	2.19	2.39	2.68
Reaction time *(min.)*	0.47	0.63	1.15
Excitement score	10.3	10.3	9.7
Thrust score	3.2	3.2	3.0
Per cent successful mounts	100.0	99.6	98.9
Volume per ejaculate *(ml.)*	2.80	2.99	2.39
Volume per week *(ml.)*	5.60	11.96	16.73
Spermatozoa per ejaculate ($\times 10^9$)	3.06	3.67	2.66
Spermatozoa per week ($\times 10^9$)	6.12	14.69	18.61
Spermatozoa per milliliter ($\times 10^9$)	1.04	1.23	1.11
Initial motility *(%)*	64.9	63.5	58.7
Abnormal spermatozoa *(%)*	13.0	11.7	11.4

[a] First ejaculates only; preliminary times for additional ejaculates were held constant at 2 min.

The libido decreased with successive ejaculates in all bulls.

Thrust scores tended to increase over the experimental period for all bulls. This was believed due to the increasing maturity of the bulls and also to their becoming accustomed to the experimental regime.

It should be noted that the longer time required to collect semen from the high-frequency bull was still of a practical magnitude for an artificial insemination organization. Whether this would continue to be true over an extended period of time can not be determined from this experiment at the present time.

Highly significant ($P = <0.01$) differences were observed between treatments on volume per unit time, volume per ejaculate, spermatozoa per unit time, and spermatozoa per ejaculate (Table 1).

The high-frequency bull produced the greatest quantity of semen and of spermatozoa per unit time, but produced a smaller quantity of semen and of spermatozoa per ejaculate than did either of his pair-mates. This increased quantity of semen becomes important in organizations which ship a large quantity of semen at any given time, or organizations which deal entirely in frozen semen. It would also have immediate application if an extender were developed which would maintain the fertilizing capacity of the semen at a high level for seven or more days.

In all cases the second ejaculate was greater in volume and spermatozoa numbers than the first ejaculate. With successive ejaculates after the second ejaculate, the volume and spermatozoa numbers per ejaculate gradually decreased.

Evaluation of initial motility ratings showed highly significant ($P = <0.01$) differences between treatments (Table 1).

The high-frequency bull produced semen of lower motility rating than did either of his pair-mates. All semen, however, was of acceptable motility for breeding purposes. Motility ratings were highest for the second ejaculate and decreased slightly for successive ejaculates.

II. The effects of varying intervals between collection periods.

Analysis of variance of the data indicated a highly significant ($P = <0.01$) difference between treatments for the preliminary time for the first ejaculate, as shown in Table 2. The high-frequency bulls required more time to react

TABLE 2

Mean values for libido and semen characteristics for varying intervals between collection periods

Characteristic	Frequency of ejaculation		
	2 every 2 days	2 every 4 days	2 every 7 days
Preliminary time[a] *(min.)*	3.43	2.36	2.21
Reaction time *(min.)*	2.86	2.02	2.32
Excitement score	9.8	10.0	10.0
Thrust score	3.0	3.1	3.1
Per cent successful mounts	96.5	96.9	98.4
Volume per ejaculate *(ml.)*	3.05	3.54	4.62
Volume per week *(ml.)*	10.51	6.17	4.61
Spermatozoa per ejaculate ($\times 10^9$)	3.96	4.72	6.48
Spermatozoa per week ($\times 10^9$)	13.85	8.25	6.57
Spermatozoa per milliliter ($\times 10^9$)	1.32	1.39	1.37
Initial motility *(%)*	64.9	63.9	66.1
Abnormal spermatozoa *(%)*	7.9	8.0	7.9

[a] First ejaculates only; preliminary times for second ejaculates were held constant at 2 min.

to sexual stimuli and produce seminal fluid from the sheath. Attempts were made to keep the preliminary time of the second ejaculate a constant 2 min.; for this reason, only the first ejaculate mean is included in Table 2.

The differences in volume of semen produced per unit time and the number of spermatozoa produced per unit time were highly significant ($P = < 0.01$) between treatments. The high-frequency bulls produced an average of 13.85 billion spermatozoa per week, compared to 8.25 and 6.57 billion for the lower frequencies, respectively.

Initial motility differences between treatments were significant at the 5% level, the low-frequency bulls having the highest motility ratings (Table 2).

Differences between ejaculates were analyzed and second ejaculates were found to be superior to first ejaculates in having lower reaction times, higher excitement thrust scores, greater volume per ejaculate and per unit time, greater numbers of spermatozoa per ejaculate and per unit time, and higher motility ratings. The number of spermatozoa produced per milliliter of semen was highest in first ejaculates. No significant difference was noted between ejaculates for the percentage of successful mounts or percentage of abnormal spermatozoa.

Generally, the reaction time tended to increase for all bulls as the experiment progressed. This could be an important factor in high-frequency collections. As can be seen in Table 2, the time required to collect the high-frequency bulls, although longer than the time required to collect lower-frequency bulls, was not excessive. Whether this would be true for bulls collected at a high frequency for a period of time greater than 2 yr. can not be determined at this time.

Volume per ejaculate and per unit time increased over the experimental period, indicating that as the bulls matured and became accustomed to their respective collection frequencies they increased semen output, regardless of those frequencies. A similar situation was observed for spermatozoa output per unit time and per milliliter.

ACKNOWLEDGMENTS

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TECHNICAL NOTES

CORRELATION OF CERTAIN BLOOD CHARACTERS IN THE HEIFER WITH MILK-PRODUCING ABILITY[1]

Criteria for the selection of dairy cattle for producing ability, in addition to the ones now available, are needed for continued improvement in dairy production. Efforts to obtain such criteria have prompted studies of body measurements, type ratings, and mammary gland development in the calf, as well as many physiological studies including thyroid status (1) of the individual. Since potential milk-producing ability is physiological potential, continued study of the physiology of the individual should yield knowledge that will be of aid in the selection of cattle for productive performance.

Many blood constituents are maintained at rather definite levels for continuance of life. These levels are maintained by a balance of various physiological mechanisms. Erythrocytes, hemoglobin and hematocrit values are maintained at a level that is peculiar for the individual calf (2). Variation among individuals may be a reflection of a difference in a dynamic balance of physiological mechanisms that bring about the individual's blood level. The level, then, may be a reflection of the physiological potential and milk-producing potential of the dairy heifer.

Since environmental factors cause fluctuations in blood characters within the individual, a number of determinations must be taken to get an estimate of the individual's characteristic blood picture. The purpose of this study was to determine whether the average of a number of erythrocyte counts, hemoglobin and hematocrit values for an individual heifer during her first year of life was associated with her future milk-producing ability.

Heifer calves in the University of Nebraska herd were bled monthly from the age of 3 mo. through 10 mo. Blood characters studied were erythrocyte count, hemoglobin and hematocrit values. Methods used for the determination of these are described by Schultze (2). The ratios of $\frac{\text{hemoglobin}}{\text{erythrocyte}}$, $\frac{\text{hemoglobin}}{\text{hematocrit}}$, and $\frac{\text{hematocrit}}{\text{erythrocyte}}$ were also studied in relation to the future milk yield of the heifers. Fifty-three Holsteins and 20 Jerseys have completed one or more lactations. The blood character taken as representative of the heifer was the average of the eight monthly determinations. Milk yield was converted to a 305-day mature equivalent and to a 3.5% FCM basis for all lactations for all cows. Average blood character level of the heifer calf was correlated with the average 3.5% FCM yield. Holstein data were grouped for analysis according to year of birth. Jerseys born in 1954 and 1955 were considered too few to divide into groups. Production in the Holsteins varied from 12,000 to 19,000 lb. of 3.5% FCM, with average erythrocyte counts from 8.2 to 11.1 million per mm.^{3} Jerseys' production varied from 8,500 to 16,000 lb. 3.5% FCM, and erythrocyte counts from 7.3 to 10.8 million per mm.^{3} The results of this study are shown in Table 1.

Although the data are limited, and animals are from one herd only, the indications are that the average erythrocyte count in heifers

[1] Published with the approval of the Director as paper No. 1033, Journal Series, Nebraska Agricultural Experiment Station.

TABLE 1

Relationship between blood characters of calves (3 through 10 mo. of age) and subsequent 305-day M.E. 3.5% FCM produced

Blood character (mean of 8 monthly determinations)	Holstein			All Holstein	Jersey	All groups combined
	Group I 19 cows	Group II 19 cows	Group III 15 cows	53 cows	20 cows	73 cows
Erythrocytes	+0.539*	+0.528*	+0.552	+0.525**	+0.536*	+0.536**
Hemoglobin	+0.059	+0.405	−0.309	+0.046	+0.158	+0.077
Hematocrit (vol. % cells)	+0.100	+0.192	+0.114	+0.131	+0.650**	+0.296
$\frac{\text{Hemoglobin}}{\text{Erythrocyte}}$	−0.481*	−0.255	−0.713**	−0.496**	−0.394	−0.397**
$\frac{\text{Hemoglobin}}{\text{Hematocrit}}$	−0.089	+0.107	−0.441	−0.118	−0.488**	−0.252*
$\frac{\text{Hematocrit}}{\text{Erythrocyte}}$	−0.420*	−0.314	−0.494	−0.404**	−0.175	−0.253*

* Significant at 5% level.
** Significant at 1% level.

is significantly related to milk-producing ability. This relationship is indicated when all eight monthly blood determinations of heifers from 3 to 10 mo. of age are used to get the representative erythrocyte count for the heifer. The use of fewer determinations (average of monthly erythrocyte counts from 3 through 6 mo. or from 6 through 10 mo. of age) showed a lower degree of correlation with milk production than the average of the eight determinations. Whether the eight samplings taken over a shorter period of life would give the same relationship is not known. The influence of season of the year may be a significant factor and this is partially eliminated by year-round determination of blood values.

Multiple correlations of erythrocyte and other constituents such as ratio of $\frac{\text{hemoglobin}}{\text{erythrocyte}}$ or $\frac{\text{hematocrit}}{\text{erythrocyte}}$, that in themselves showed indication of being related to milk production, gave only slightly higher multiple correlation coefficient values than erythrocyte level alone.

The results presented here are necessarily of a preliminary nature because of the limited data. However, the consistency of significant correlation of the different groups may indicate a real relationship between average erythrocyte count of the heifer and her potential milk-producing ability.

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AN EMULSIFIED SOYBEAN OIL FOR BLOAT THERAPY [1]

Frothing of ruminal contents now is accepted quite generally as the immediate cause of pasture bloat (2). This view is supported by the effectiveness of anti-foaming agents in preventing and treating bloat. Reid and Johns (6) effectively treated bloat in cattle on red clover pasture with vegetable oils (peanut, raw linseed, soybean, and olive), vegetable turpentine, emulsified tallow, whale oil, cream, lanolin, liquid paraffins, and paraffin-wax emulsions. Silicones and some detergents were found to be unreliable, although a recent report (5) indicates that certain detergents are effective in prevention and treatment. In previous work at this station (1, 3, 4), a water-dispersible lard oil[2] was found to be effective in treating serious cases of bloat. Moreover, relief was more prompt than with unemulsified oils. Since lard oil is not available for general use in bloat therapy, the development of other effective products seemed desirable. Also, the possible use of comparatively lower cost ingredients appeared promising. The objective of the present study was to develop an inexpensive, nonirritating, water-dispersible product with rapid, efficient bloat-relieving action. The development and testing of such a product is described herein.

Soybean oil was chosen as the basic antifoaming ingredient because of its availability, relatively low cost, and palatability. It is recognized, however, that other oils might be satisfactory, with possibly some modification in composition of emulsifier and/or method of preparation of the product.

Several emulsified products were prepared for preliminary investigations. Emulsion No. 4 (E-4) was judged most suitable (on the basis of the criteria stated in the objective). In preparation of the emulsifier, 3.8 g. of casein was soaked in water for several hours, being stirred constantly with a magnetic stirrer. Then, the following ingredients were added successively: 2.9 g. of anhydrous sodium carbonate to disperse the casein, 220 ml. of 95% ethanol, and sufficient water to bring the total volume to 725 ml. The final product (E-4) was prepared by mixing one part of this emulsifier, v/v, with two parts of crude soybean oil. The lecithin in the crude soybean oil appeared to be an essential feature for satisfactory emulsification. Occasionally, after standing for a prolonged period, some stratification occurred; however, the mixture re-emulsified readily when shaken. Mechanical homogenization essentially prevented stratification but seemed to have little effect on the efficacy of the product as a bloat treatment. Batches of this mixture were prepared periodically as needed; thus, development of rancidity was not a problem. It would seem, however, that addition of an antioxidant to prevent deterioration of the oil during prolonged storage would be an appropriate modification.

Results of field tests in 1959 with E-4, when used in treating bloated cattle, are shown in Table 1. Of 95 cases of bloat with a severity of three or greater (4) at the time of treatment with E-4, 90 recovered promptly, four

[1] Journal Paper No. J-3886 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1267.
[2] Supplied by Midwest Dried Milk Co., Dundee, Illinois.

TABLE 1

Responses of serious cases of bloat in cattle to treatment with a water-dispersible soybean oil (E-4)

Bloat severity[a]	Level	Bloat cases			
		Treated	Recovered promptly	Died	Other treat.[b]
	(ml. agent)				
3	100	3	3		
	150	2	2		
	200	27	26		1
	250	10	10		
	300	45	42		3
	400	1	1		
4	200	1	1		
	300	4	4		
5	150	1	1		
	200	1		1	
		95	90	1	4

[a] According to scale of Johnson *et al.* (4).

[b] Required 100-200 ml. additional E-4 about 20 min. after initial treatment to effect recovery.

required later treatment with the same agent, and one died during the initial stage of treatment. It must be recognized that spontaneous recovery sometimes will occur even in seriously bloated animals. In the present study, however, a stomach tube was used first in an effort to relieve the bloat. Any cases which could be relieved in this manner were not included in the data. When no relief could be provided by the stomach tube alone, E-4 was immediately introduced by stomach tube. This was followed, usually in less than a minute, by release of a large volume of gas via stomach tube and/or eructation. In most cases, animals were completely deflated and resumed grazing within 15 to 40 min.

Unemulsified soybean oil, although moderately effective when used in other contemporary bloat cases, often did not result in prompt relief and was judged less suitable than the emulsified product. No concurrent comparisons were made in cattle between lard oil and the emulsified soybean oil; however, on the basis of results in different years the two preparations appeared to be equally effective (4).

In other studies in 1959, lambs grazing alfalfa pasture were drenched (by dosing syringe) with 1 to 1.5 oz. of either lard oil or E-4 when they were bloated seriously. In most cases the drenching alone was sufficient to relieve symptoms, but in a few cases, use of a stomach tube was necessary. In most cases either oil treatment relieved the lambs in 20 to 30 min.; in many cases the relief was almost immediate. Results are summarized in Table 2.

TABLE 2

The effectiveness of lard oil and E-4 as a treatment for bloat in lambs

	Lard oil	E-4
Cases treated and not scored afterward		
No. of cases	46	26
Av. bloat scores at time of treatment[a]	3.3	3.5
No. cases requiring stomach tube	5	0
Cases treated and scored later		
No. of cases	29	13
Av. bloat score at time of treatment[a]	3.1	3.8
Av. bloat score approximately 30 min. later[a]	1.1	1.1
No. cases requiring stomach tube	3	0

[a] According to the scale of Johnson *et al.* (4).

These data indicate that the product reported herein (E-4) meets the criteria outlined earlier in this paper; it is an inexpensive, nonirritating, emulsified product with rapid, efficient bloat-relieving action.

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CHANGES IN REPEATABILITY WITH CHANGES IN HERD ENVIRONMENT

In many situations a decrease in environmental variance resulting from a reduction of temporary environmental differences among individual herd members would seem logically to accompany improvement in herd environment. Such an effect would be reflected in increased estimates of repeatability and heritability. In recent years there has been increasing interest in the effect of level of environment on the heritability of various quantitative traits. Considerable evidence has accumulated to the effect that the success of selection for certain economic traits in farm animals is greatly dependent on the environment in which selected individuals develop and the environment in which they are used as breeding stock. A recent interesting example of the effect of environmental level on variability through an influence on fitness traits has been presented by Vetukhiv and Beardmore (1).

The present note pertains to a statistical study of 1,564 lactation records from 523 cows (262 Jerseys and 261 Holsteins) belonging to 17 birth-year groups in the herds of the Agricultural and Mechanical College of Texas and the Texas Agricultural Experiment Station. Intra-class correlations were computed in the usual manner as estimates of repeatability of milk production, fat production, and fat test. Estimates for the entire period for the three traits were 0.44, 0.39, and 0.69, respectively. Since it was known that definite improvements in various aspects of herd management were initiated at a rather precise point in time, the records were divided into two groups, those made before and those made after this point, and separate estimates of repeatability were calculated from each segment of the data. The first period included records from 14 birth-year groups, and the second included records from three. The estimates for the first period were 0.37, 0.32, and 0.70, while the corresponding ones for the second period were 0.49, 0.44, and 0.71. These changes in the estimates for milk and fat production were probably due, at least in part, to a reduction in temporary environmental differences among herd members, which automatically accompanied the improvement in average environment. Sampling errors, which were not assessed at the time the study was made in 1951 by one of us (Stone) as a Master's thesis project, would probably be somewhat larger for the estimates from the second period because of the much smaller number of records represented (202). Neither were the differences between the two sets of estimates tested statistically. However, it seems likely that repeatability was truly larger for milk and fat production during the second period. It seems likely also that heritability increased, though sire groups were too few and too small to yield reliable estimates.

Perhaps there are in existence data from larger herds in which relatively sudden rather than gradual changes in environment are known to have occurred. It would be interesting to know if marked changes in repeatability and heritability occurred in such herds following the environmental changes. It is conceivable that such changes, whether improvements or deteriorations such as sudden increases in the incidence of chronic disease conditions, could cause various combinations of increases and decreases in total variance, environmental variance, and genetic variance.

Current interest in the influence of environmental level on the effectiveness of selection and in genotype-environment interaction, and the possibility of investigating these points with existing data, seemed to justify calling to readers' attention this suggestion of changing repeatability in the Texas data.

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MILK LIPIDS SYMPOSIUM[1]

STUART PATTON, Chairman

INTRODUCTION

Milk fat is one of the most palatable food components known to man. In the form of milk and various dairy products, and as an ingredient in cookery, it is widely consumed by all age groups. The demand for milk fat has been such as to establish selective breeding of cows for high fat production as a standard practice. It can be stated with the greatest confidence that the number of milk samples tested for fat content has been astronomical, but until recently milk fat composition and properties was a consideration of interest to a very few research workers. This inclination to let the cow do what she will regarding qualitative aspects of milk fat production is open to question for two reasons. First, it seems doubtful that further improvement can be made in various dairy products, on the assumption that composition and properties of milk fat are of little importance. Secondly, it seems unwise to ignore the current concern that many have for their weight and the state of their arteries. This concern has provoked a tremendous research effort in the field of lipid metabolism. Although the dairy industry is not oblivious to these considerations, its research program on milk lipids seems somewhat inadequate. A mastery of milk fat synthesis, composition, and properties would at least be good insurance for the future; conceivably, it could be the basis of better and more healthful dairy products. The milk fat that makes for ideal texture in butter may not necessarily yield optimum dispersibility and flavor in dry whole milk. With respect to nutrition, the milk fat consumed by infants, normal adults, and elderly heart-patients may well represent three entirely different requirements from the standpoint of fatty acid composition.

Thus, as a Symposium topic, Milk Lipids seems appropriate and timely. The objective of the Symposium, stated simply, is to examine how milk fat is made, how its composition can be analyzed, how it may be metabolized in the body, and how it may deteriorate prior to consumption. In the limited time available the treatment of these subjects can hardly be exhaustive. However, it is hoped that the papers of this Symposium, together with other papers on milk lipids to be presented at this meeting, will indicate the frontiers of knowledge on the subject.

[1] Presented in a Symposium at the American Dairy Science Association's meeting, Utah State University, Logan, June 20, 1960.

MILK FAT SYNTHESIS

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The origin of milk fat in general, and of the short chain fatty acids in particular, has resisted the investigations of scientists for more than half a century—even though their labors have been fruitful. Nevertheless, the theory of milk fat synthesis most widely accepted today differs only in degree of refinement from that proposed by Carlo Foà in 1912 (8), despite the development of chromatography, radioactive isotopes, the Warburg apparatus, and the like. This is not to imply that Foà's experiments and techniques left little to be desired nor that his work was overlooked by his successors. On the contrary, nearly everyone has credited Foà with being the first to use perfused udders for the study of synthesis of milk. However, it seems Foà's contributions were more significant than that. He insisted that the udder plays an active role in the synthesis of milk fat, absorbing certain triglycerides from blood and rejecting others. More important, he stated that the short chain fatty acids originate in the udder and that they are synthesized from noncarbohydrate sources. Further, Foà was apparently the first to state that lactose is synthesized in the udder from plasma glucose.

Foà apparently failed to convince his colleagues and successors of the validity of his observation, however, for a number of papers between 1934 and 1940 supported the idea that milk fat is synthesized entirely from plasma lipids (13, 25, 30, 46). This idea fitted nicely with Hilditch's observations on the fatty acid composition of ox blood and milk fat triglycerides, and in 1937 he proposed that the short chain fatty acids of milk fat resulted from a stepwise omega degradation of the long chain fatty acids, especially oleic, which were a part of the absorbed triglycerides (14). Hilditch's theory was well founded, and undoubtedly would be widely accepted today had not the Atomic Age made radioactive tracers available.

The first experimental evidence to indicate that the short chain fatty acids might arise by

an entirely different pathway came from the Russian workers Asimoff and Kashevaroff [cited by Nikitin, (34)] who, in 1940, reported that the mammary gland absorbs significant quantities of *beta*-hydroxybutyric acid from the plasma. Shaw and Knodt (44) confirmed this finding in 1941 and calculated that the *beta*-hydroxybutyrate absorbed was more than enough to account for the synthesis of all the milk fatty acids from C_4 through C_{14}.

Experimental work in ruminant metabolism was scant during the war years. In 1943, however, a paper by Barcroft and coworkers (2) suggested that the volatile fatty acids in milk fat might arise the volatile acids produced in the rumen. That suggestion apparently stimulated subsequent research. Immediately after the war, a large number of relevant articles appeared in the English journals. The volatile fatty acids found in the rumen and peripheral blood of cattle and sheep were identified as acetate, propionate, and butyrate (3, 6, 7, 19, 29, 32, 40, 41), and in 1949 G. L. McClymont (31) demonstrated that large amounts of acetate are absorbed by the mammary gland of the lactating cow.

C^{14}-labeled metabolites became available for biological research during this period, and almost immediately thereafter Popják, Folley, French, and their coworkers in England [see Folley (9)], and Kleiber and coworkers at Davis, California (20, 21, 22), proved beyond reasonable doubt that the circulating volatile acids in the blood are indeed important precursors of milk fat.

LIPID PRECURSORS OF MILK FAT

Comparison of dietary fat with milk fat. Figure 1 compares the fatty acid composition of pasture clover lipids with milk fat and emphasizes the point that large changes must occur in the portion of dietary fat that eventually becomes a part of milk fat. This is not to imply that milk fat may be derived entirely from dietary fat; one can readily calculate that the average milk cow secretes about twice as much fat in milk as she eats in her feed. In other words, the cow must synthesize at least half her daily production of milk fat from nonlipid sources. The figure also shows that clover lipids contain large amounts of unsaturated fatty acids—oleic, linoleic, and especially linolenic, the last being almost completely absent in milk fat. In contrast, milk fat contains relatively large amounts of short chain fatty acids—acids which are not found in appreciable quantities in any other lipid fraction of the cow's body.

Effect of rumen microflora on dietary lipids. Figure 2 shows the fatty acid composition of lipids taken from rumen contents of sheep that were grazing on clover-rich pastures (Figure 1). Comparison of these two figures indicates that the dietary lipids undergo extensive change

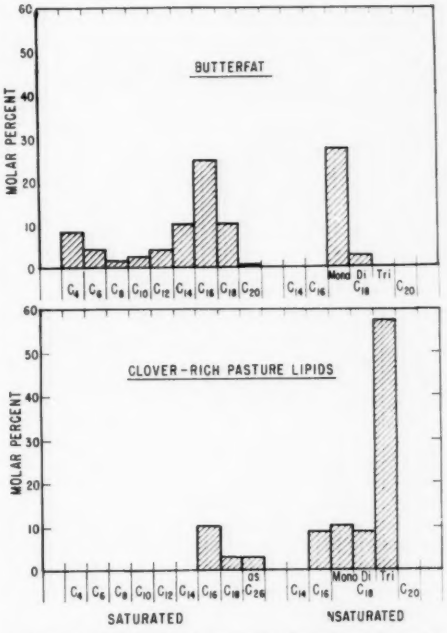

FIG. 1. Fatty acid composition of clover-rich lipids [data of Shorland *et al.*, Reference (47)] compared with that of butterfat [Hilditch, Reference (15)].

even in the first step of digestion. Shorland *et al.* (47) were attempting to explain why prolonged feeding of highly unsaturated oils failed to decrease the degree of saturation of body depot fat. Dietary lipids contain large proportions of C_{18} unsaturated fatty acids, particularly linolenic acid, but very little stearic acid. The lipids isolated from rumen

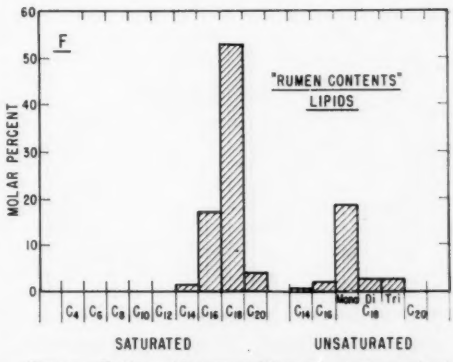

FIG. 2. Fatty acid composition of rumen-content lipids taken from sheep eating clover-rich pastures [data of Shorland, Reference (47)].

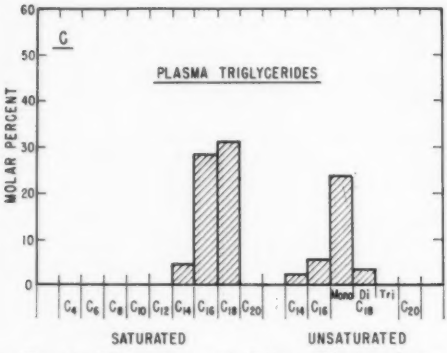

FIG. 3. Molar per cent fatty acids in plasma triglycerides of Ayrshire cattle. [Data of Lough and Garton, Reference (26)].

contents, in contrast, contained very little linolenic acid but large amounts of oleic and stearic acids. These observations led to the conclusion that the highly unsaturated dietary acids are extensively hydrogenated in the rumen. Ruminal hydrogenation also accounts for the presence of *trans* isomers of fatty acids in depot and milk fat.

Comparison of rumen content lipids with blood lipids. The fatty acid composition of the plasma triglycerides is shown in Figure 3, where it may be seen that except for minor differences, there is a rather close similarity in molar percentage of fatty acids in this fraction of the plasma lipids and the lipids in the rumen. In contrast, the molar percentage of fatty acids in the plasma sterol esters (Figure 4) differs considerably from that of rumen content lipids. Since cholesterol esters of bovine plasma make up nearly 80% of the total lipids, they must be considered as possible precursors of milk fat. Cholesterol esters

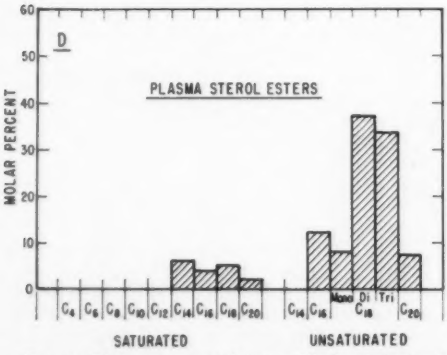

FIG. 4. Molar per cent fatty acids in cholesterol esters of Ayrshire cattle [data of Lough and Garton, Reference (26)].

typically contain large amounts of C_{18} unsaturated fatty acids, and in this respect resemble dietary lipids more closely than depot or milk fat lipids. This observation, plus the little cholesterol in the diet of dairy cows (most of it is synthesized endogenously), led Lough and Garton (26) to propose that cholesterol is esterified with long chain fatty acids of dietary origin, either during or after their absorption from the gut. In other words, this may be viewed as a transport mechanism for the dietary unsaturated fatty acids.

Composition of blood lipids. Figure 5 was

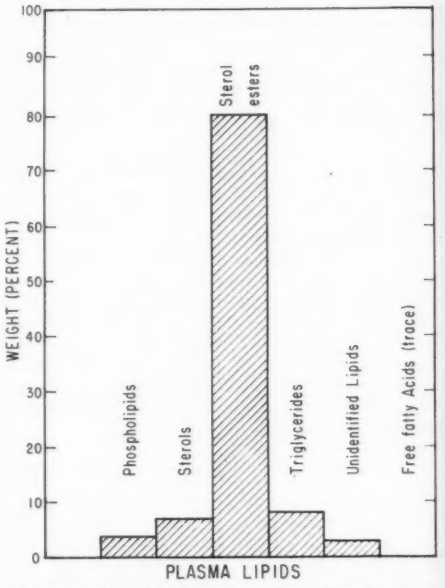

FIG. 5. Weight per cent of various lipids in Ayrshire blood [data of Garton and Duncan, Reference (11)].

taken from the work of Garton and Duncan (11), who used silicic acid column chromatography to separate the various blood lipids of Ayrshire cows. This figure shows that approximately 80% of the circulating lipids are cholesterol esters, and that there are 7.5% triglycerides, 6.5% sterols, 3.8% phospholipids, 2.5% unidentified lipids, and only traces of free fatty acids. Milk fat, considerably different from plasma lipids, contains about 98% triglycerides, 1% phospholipids, and less than 1% cholesterol esters (17). Studies of the uptake of various plasma lipids by the mammary gland have been conducted over the past 50 yr. by determining the difference in concentration of a particular lipid fraction in the arterial blood supply to the udder and in the venous drainage from the udder. There

seems to be unanimous agreement that the mammary gland absorbs large quantities of triglycerides from the blood. Indeed, several workers have estimated that the uptake of these glycerides is sufficient to account for the complete production of milk fat (13, 30, 46).

There is some disagreement on whether phospholipids and cholesterol esters are used for fat synthesis. Nikitin (34) claims work in Russia during the war demonstrated that up to 40% of milk fat is derived from phospholipids of the blood. This idea seems to be supported by two recent reports by Riis, Luick, and Kleiber at Davis (42), and by Glascock and his group in Reading (12). Even so, arterio-venous difference studies have consistently failed to show a significant uptake of either cholesterol esters or phospholipids by the mammary gland of dairy cattle, and there seems to be no experimental proof that either of these two lipids plays an important role in the net transport of fatty acids in other species (10).

Comparison of milk fat with depot and blood fat. The fatty acid composition of depot fat

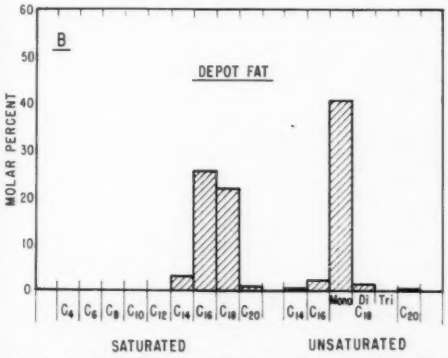

FIG. 6. Molar per cent fatty acids in depot fat of cattle [data of Hilditch and Longenecker, Reference (16)].

is shown in Figure 6. Depot fat contains large amounts of palmitic, stearic, and oleic acids and relatively few short chain fatty acids. In contrast, milk fat contains large amounts of short chain fatty acids and relatively little stearic and oleic acid. It may be of some interest to recall that changes in animal diet and health (18, 43) result in large changes in the fatty acid composition of milk fat but have little effect on the composition of depot fat. The principal difference in fatty acid composition between plasma triglycerides and milk fat (Figures 1 and 3) is due to the presence of C_4 through C_{14} fatty acids in milk fat; the plasma glycerides contain relatively more palmitic, stearic, and C_{14} and C_{16} unsaturated fatty acids. Comparison of Figures 1 and 4 shows that the difference in fatty acid composition between plasma sterol esters and milk fat is even more striking—particularly in the large amounts of unsaturated fatty acids in the sterol esters. As mentioned earlier, there is little evidence that the udder absorbes cholesterol esters from the plasma; comparison of the fatty acid moieties of these two lipids shows that even if sterol ester fatty acids were available for milk fat synthesis, they would have to undergo extensive chemical change before they could become a part of the milk fat molecule.

These comparisons have shown that lipids that might be considered obligatory intermediates in the synthesis of milk fat differ considerably from one another in fatty acid composition. Blood lipids have been fractionated into four or five components, and each of these fractions has, at one time or another, been considered a possible precursor of milk fat. It now seems positive, however, that only the blood triglycerides are absorbed in quantities large enough to contribute significantly to milk fat synthesis. They have also shown that milk fat differs considerably in fatty acid composition from all other lipids in the ruminant body, particularly in regard to the presence of short chain fatty acids. Intensive metabolic activity by ruminal microorganisms results in the production of *trans* isomers of the dietary fatty acids. Unsaturated fatty acids undergo hydrogenation and, more important, large quantities of short chain fatty acids, especially acetate, propionate, and butyrate are synthesized from nonlipid dietary precursors.

NONLIPID PRECURSORS OF MILK FAT

The bulk of the acetate produced in the rumen seems to pass unchanged through the liver and into the general circulation (29). On the other hand, under normal dietary regimes, most workers now hold that little propionate or butyrate, as such, finds its way into the peripheral blood (3, 19, 29, 41). Propionate is thought to contribute most of its C to the synthesis of glucose (22, 32, 40). Butyrate is oxidized to *beta*-hydroxybutyrate by the ruminal epithelium and perhaps by the liver (1, 35, 48, 49). Some butyrate may escape into the general circulation, but one may readily calculate from arterial blood butyrate concentrations and blood flow through the udder that butyrate as such—even if completely absorbed and used exclusively for fat synthesis—can not contribute more than 13% of the C necessary for the synthesis of short chain fatty acids.

SYNTHESIS OF MILK FAT IN THE MAMMARY GLAND

The synthesis of milk fat in the mammary gland is not completely understood. The long chain fatty acids presumably come directly from the triglycerides which are absorbed from

the blood, and there is little evidence to suggest that other plasma lipids are important precursors of milk fat. It is now almost certain that the short chain fatty acids are synthesized from acetate and *beta*-hydroxybutyrate (5, 21, 24, 37, 38) according to the following scheme.

Beta-hydroxybutyrate is absorbed into the udder (36, 44) and follows one of two major pathways: reduction to butyrate or cleavage between the second and third C atoms to yield methyl and carboxyl 2 C fragments. The carboxyl 2 C fragment seems to be used preferentially for fatty acid synthesis, and presumably follows the same pathway as acetate (24, 45). Head-to-tail condensation of these 2 C fragments is thought to account for 40% of the newly synthesized butyrate; the remaining 60% comes from direct reduction of the newly synthesized *beta*-hydroxybutyrate (38). Subsequent chain elongation, brought about by the stepwise addition of 2 C units on to the carboxyl end of the fatty acid, ultimately leads to the synthesis of all acids up to C_{10} and perhaps even to C_{16} (24, 38).

ORIGIN OF MILK FAT GLYCEROL

The absorption of triglycerides from blood necessarily implies the absorption of equimolar quantities of glycerol. Since sufficient triglyceride is absorbed to account for the complete synthesis of milk fat, one might assume the same applies to glycerol. The fallacy of this logic is well known and applies to nearly all statements which are based on absorption studies. The absorption of a substance does not necessarily mean the synthesis of a chemically similar substance. Moreover, the absorbed substance might be catabolized or used for the maintenance of structure and function within this very active tissue. The origin of milk fat glycerol seems to be a case in point.

Figure 7 compares the radioactivity in milk fat glycerol with plasma glucose following the intramammary infusion of uniformly C^{14}-labeled glucose (28). It also shows the summated specific activity of C^{14} in milk fat glycerol and plasma glucose. The ratio of these two terms is known as the transfer quotient (23) and expresses the importance of glucose as a precursor of glycerol. In this trial, it indicates that 68% of milk fat glycerol is derived from plasma glucose.

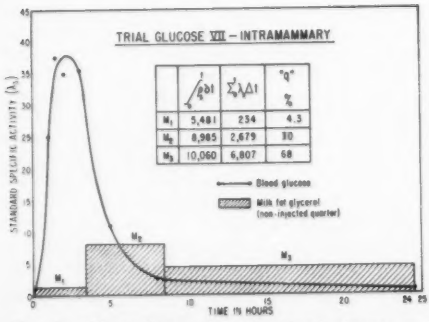

FIG. 7. C^{14} specific activity in blood glucose and milk fat glycerol after the intramammary infusion of U–C^{14}–labeled glucose [data of Luick and Kleiber, Reference (28)].

Although this calculation shows that a large fraction of milk fat glycerol comes from glucose, it tells nothing of the two pathways which might be involved: The synthesis of glycerol from glucose in the udder or the synthesis of plasma lipid glycerol from glucose, presumably in the liver (4), and its subsequent absorption into the udder. Recent work in our laboratory (27), shown in Figure 8, indicates that glycerol

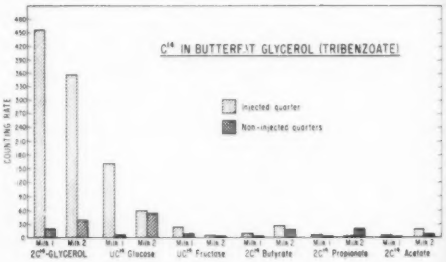

FIG. 8. C^{14} in milk fat glycerol following the intramammary infusion of 2C^{14} glycerol, U–C^{14} glucose, U–C^{14} fructose, 2C^{14} butyrate, 2C^{14} propionate, and 2C^{14} acetate.

synthesis from glucose does, indeed, occur in the udder. This figure compares the radioactivity in glycerol taken from the infused and noninfused quarters of lactating cows following the intramammary infusion of C^{14}-labeled glycerol, glucose, fructose, butyrate, propionate, and acetate. Only glucose and glycerol lead to high labeling in milk fat glycerol and in these two trials glycerol isolated from the infused quarter is much more radioactive than glycerol taken from the noninfused quarters. Glycerol per se can not be considered an important precursor of milk fat glycerol since there is little, if any, free glycerol in the blood of cattle. However, the high labeling after the infusion of C^{14}-labeled glycerol is of unusual interest, since it implies that glycerol, if synthesized in the udder, becomes a part of the milk fat molecule.

In contrast, large quantities of glucose are absorbed in the udder (13, 33) and, therefore, the high labeling in milk fat glycerol after the infusion of C^{14}-labeled glucose means that glycerol is synthesized in the udder. In this regard, our results confirm earlier findings with rabbits (39) and cows (50).

These results suggest a new concept of milk fat synthesis. Up to now we have generally

conceived of milk fat synthesis as a rearrangement or substitution of fatty acids on preformed triglyceride molecules which are absorbed from the blood. The synthesis of glycerol from glucose in mammary tissue and the subsequent incorporation of glycerol into milk fat means that milk fat, to an unknown extent, must be synthesized de novo within the mammary gland from glycerol and free fatty acids.

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GAS CHROMATOGRAPHIC ANALYSIS OF MILK FAT [1, 2]

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Gas chromatography is one of those remarkable developments in science with far-reaching effects. Primarily, it is a means of separating mixtures of volatile organic compounds in a highly efficient manner.[3] This principle is of such general importance that it is difficult to predict the many ultimate uses that gas chromatography may have. Although broad exploration of the method began about 5 yr. ago, it is already evident that gas chromatography will be indispensable in lipid analysis. At the moment, it is most appropriately applied in determining fatty acid composition of lipids through analysis of the methyl esters. Its use in the analysis of steroids and intact triglycerides is being probed in a number of laboratories. Although vapor pressures of such classes of compounds are discouragingly low at reasonable temperatures, some members of these groups have been gas chromatographed satisfactorily.

To date, milk lipids have not received extensive appraisal with this new analytical tool. A recent report (6) from our laboratory reviewed the limited literature and explored qualitative and quantitative problems in gas chromatographic analysis of fatty acid methyl esters of milk fat. In brief, the study showed that such analysis is feasible and that gas chromatography holds much promise in application to

[1] Authorized for publication as Paper No. 2466 on June 17, 1960, in the Journal Series of the Pennsylvania Agricultural Experiment Station.
[2] Supported in part by the U. S. Public Health Service (H–3632).
[3] The theory and practice of gas chromatography has been the subject of so many lectures, papers, books, and instrument instruction manuals that it has been omitted from the published version of this paper.

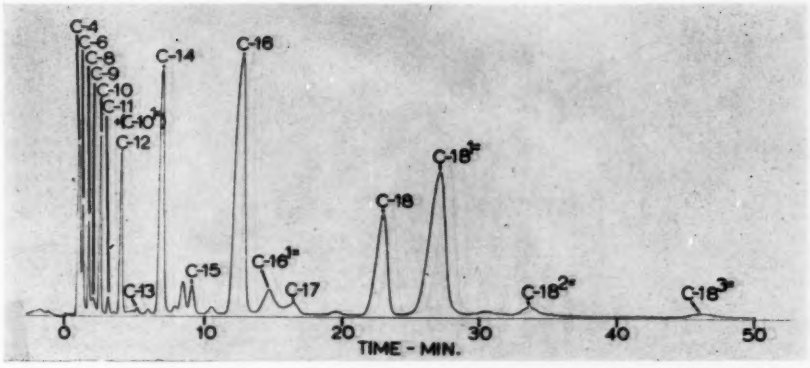

FIG. 1. Separation of 0.2 μl. of methyl esters from milk fat (November) on 9-ft. by 1-mm. column containing 40% diethylene glycol succinate polyester on 100–140 mesh Celite. Temperature—184° C., argon pressure—30 p.s.i., voltage—750, sensitivity—3×10^{-8} amp.

problems of milk lipids. These initial results have been extended to include some preliminary findings on analysis of a milk fat fraction as such, and further observation on methyl ester analysis. All of the data reported are from argon-ionization detection instruments (Barber-Colman Models 10 and 20).

Methyl ester analysis. With the exception of methyl butyrate, the major fatty acids in milk fat pose no problem in analysis as methyl esters. Methyl butyrate does not seem to respond to detection quite linearly with the other esters, and when solvents are used it is merged in the solvent front (6). However, the minor components in the C_{10} through C_{16} range, especially those with branched chains, odd carbons, and double bonds, do present problems. Figure 1 shows a typical separation of milk fat esters on a packed, polyester column. The oleate-stearate separation is very clean, but the bunching of minor components between the C_{12} and C_{16} peaks is evident. In this connection, the merits of a capillary column (200-ft. Apiezon-L) can be seen in Figure 2. The minor components and even the *cis-* and *trans*-C_{18} monounsaturated acids esters are quite well separated. However, the C_{18} di- and tri-unsaturated acid esters (linoleate and linolenate) remain unseparated and what appears to be the C_{10} monounsaturated component (9-decenoate) is only partially resolved in the front of the C_{10} peak. Some impression of comparative performance between 100- and 200-ft. Apiezon-L capillaries can be gathered from Figures 2 and 3. Total theoretical plates for these two columns were calculated to be 12,000 and 46,000, respectively, at the C_{18} peak.

Figure 3 is actually a chromatogram for a sample of completely hydrogenated milk fat. Room temperature hydrogenation was effected as previously described (2). The blackened areas were imposed on the chromatogram from

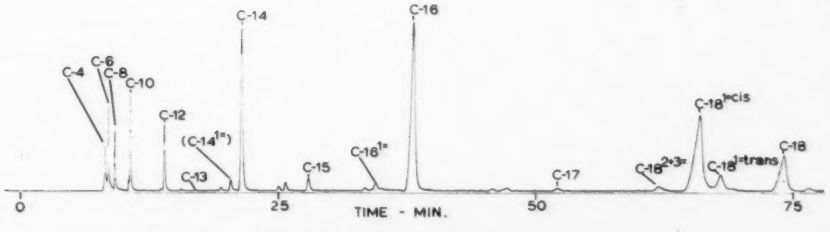

FIG. 2. Separation of 0.01 μl. of methyl esters from milk fat (September) on a 200-ft., 0.01-in. i.d. capillary coated with Apiezon-L. Temperature—212° C., argon pressure—20 p.s.i., voltage—1,700, sensitivity—1×10^{-7} amp.

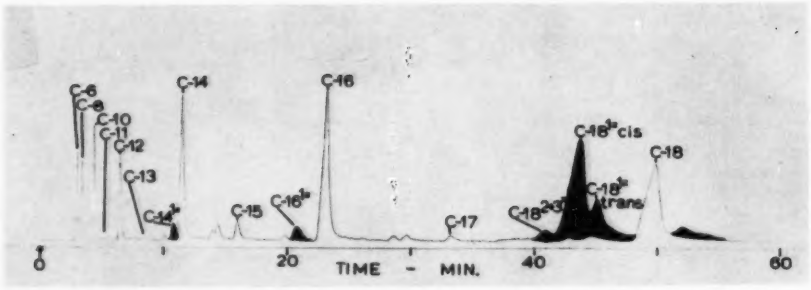

FIG. 3. Separation of 0.005 μl. of methyl esters from fully hydrogenated milk fat (September) on a 100-ft., 0.01-in. i.d. capillary coated with Apiezon–L. Temperature—213° C., argon pressure—15 p.s.i., voltage—1,700, sensitivity—3×10^{-8} amp. The blackened areas are those removed by the hydrogenation.

a tracing for the unhydrogenated fat. The samples were also analyzed on a diethyleneglycol succinate packed column (data not shown.) The purpose of the experiment was to determine which of the minor peaks on the chromatograms arose from unsaturated acids. The results suggest that from the C_{10} up to, but not including, the C_{18} components there are only four peaks attributable to unsaturated compounds. These correspond to the C_{10}, C_{12}, C_{14}, and C_{16} monounsaturated acid esters. Thus, most of the minor peaks arise from component acids with branched chains and odd numbers of carbons. The peak following C_{18} (stearate) in Figure 3 is an enigma. It arises from an unsaturated ester, but in theory it corresponds to no known compound unless it would be an all-*trans*, polyunsaturated C_{18} acid ester.

Triglyceride analysis. While the presence of short chain fatty acids (C_4 through C_{10}) in milk fat makes gas chromatographic analysis of the methyl esters somewhat difficult, their presence encourages exploration of the intact triglycerides. The quantity of these acids in milk fat makes it theoretically probable that some relatively low molecular weight triglycerides are present and such glycerides should be the ones most readily approached by gas chromatography. For example, tributyrin, tricaproin, and tricaprylin are quite easily gas chromatographed. As shown in Figure 4, a log plot of their retention times against total numbers of fatty acid carbons yields a straight line. The steep slope of this line indicates that it would take days for tristearin to emerge from the column under the operating conditions (see legend, Figure 5). Although it is possible to manipulate the variables involved, in such a manner that individual triglycerides such as trilaurin can be moved through a short packed column in a reasonable period of time, such measures actually aggravate the problem of investigating a range of naturally occurring triglycerides. Thus, at the moment, the higher molecular weight triglycerides seem out of reach by gas chromatography and we

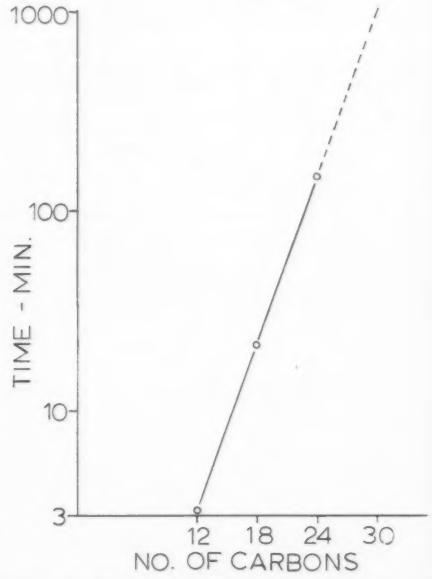

FIG. 4. The relation between retention time in a gas chromatography column and total numbers of carbons in the fatty acids of tributyrin (C_{12}), tricaproin (C_{18}), and tricaprylin (C_{24}).

have confined our efforts to lower members. Low temperature crystallization of milk fat from acetone tends to concentrate glycerides containing short chain and unsaturated acids in the filtrate. Such filtrates were secured at −70° C. and freed of solvent as described by Mattick *et al.* (2). Low-melting milk fat thus obtained was gas chromatographed under a wide variety of temperatures, pressures, column lengths, and coatings. The best results to date are presented in Figure 5, which portrays a 500-min. run on a 200-ft. Apiezon-L capillary at 250° C. and 30 p.s.i. argon pres-

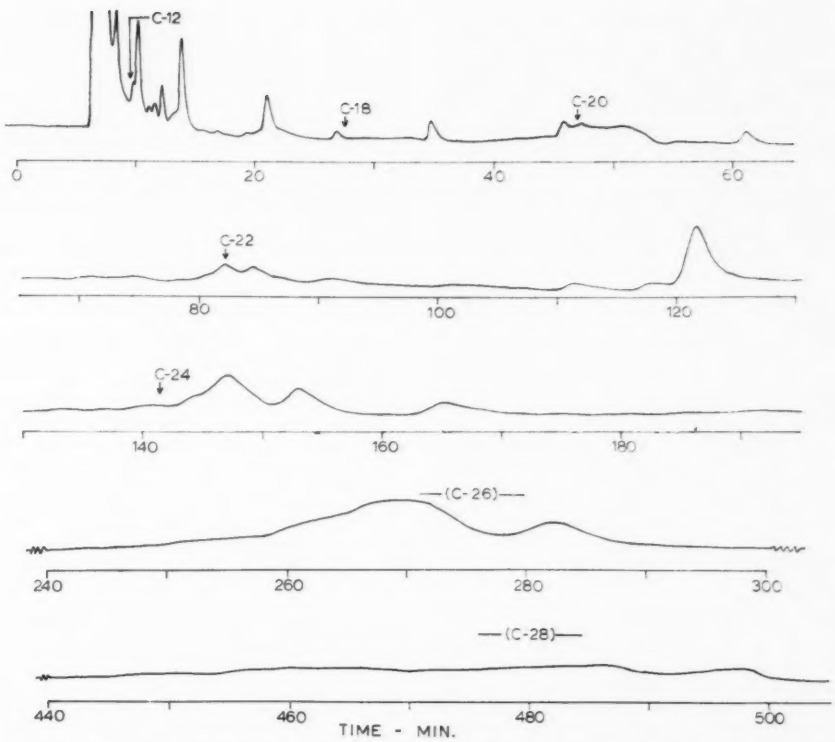

FIG. 5. Separation of 1.0 μl. of a low melting milk fat fraction (April) on a 200-ft., 0.01-in. I.d. capillary coated with Apiezon–L; Temperatures: column and detector—250° C., sample heater—285° C.; argon pressure—30 p.s.i.; voltage—1,700; sensitivity—3×10^{-8} amp. Approximate emergence positions for various triglycerides are indicated by total fatty acid carbon numbers.

sure. The emergence positions for tributyrin, tricaproin, and tricaprylin are shown (C12, C18, and C24, respectively). By use of a plot of these three triglycerides (Figure 4), the emergence times for triglycerides containing various numbers of carbons can be approximated and these also are shown on Figure 5. Two portions of the scan, from 195 to 240 and from 300 to 440 min., showing nonvarying baseline, have been omitted. Although as data Figure 5 leaves much to be desired, it does give evidence of triglyceride fractions. Because of the resolving power of gas chromatography, these fractions probably are being seen in better detail than they ever have before. Other data reinforce the following points interpreted from Figure 5: The lowest molecular weight triglyceride fraction occurring to a significant extent in low melting milk fat contains, in the aggregate, 20 carbons in the fatty acids. The amounts of triglycerides of lower molecular weight in such milk fat are essentially, if not absolutely, nil. Starting with the 20 carbon fraction there is evidence of additional fractions which seem to increase in molecular weight by two carbon units. These fractions appear to increase in quantity as they increase in molecular weight. These observations suggest that there are few, if any, triglyceride molecules in milk fat which do not contain at least one fatty acid comprised of 12 or more carbons. We have on repeated occasions attempted to demonstrate tributyrin and tricaproin in milk fat by various experimental approaches. None of these attempts has succeeded.

There are numerous unidentified peaks in Figure 5. These apparently are not triglycerides, since they are largely removed from that fraction by silicic acid chromatography.

Cholesterol. When normal milk fat (5 μl.) is gas chromatographed under the following conditions, one major peak that corresponds precisely in retention time (24 min.) with authentic cholesterol can be seen. The conditions are: 6-ft. by 5-mm. column packed with 60-80 mesh glass beads coated with 0.1% Api-

ezon-L; column temperature 278° C., flash heater 310° C., detector 295° C.; cell voltage 1,000, argon pressure 30 p.s.i., and recorder sensitivity 1×10^{-7} amp. full scale. Under these conditions, authentic cholesteryl acetate emerges in 21 min. and gives a somewhat sharper peak than does cholesterol.

In our attempt to show the usefulness of gas chromatography in milk lipids analysis, application to somewhat academic problems of lipid structure has been emphasized. However, the method also can have practical value. As an example, the possible impact of fast, detailed analysis of milk fat for fatty acid composition may be considered. Older vacuum distillation methods require days in highly skilled hands for such an analysis. The number of samples that can be accommodated by such methods is decidedly limited. Gas chromatography makes feasible the analysis of milk fat samples from many animals. Indeed, it is quite possible that such analysis may represent for milk fat composition what the Babcock test has been to milk fat production. Cows can certainly be bred and fed to produce milk fats with some variation in composition. At this point, we need to know how great this variation can be and what the merits of the resulting fats will be with respect to milk products, quality and nutritive value.

Finally, we might consider how academic a subject is the structure of milk fat. Gas chromatography has already given some indication of the specific positioning of fatty acids in milk fat triglycerides (4, 5). This approach to lipid structure has been made possible by the discovery that pancreatic lipase preferentially releases fatty acids esterified in the 1 and 3 positions of triglycerides (1). If such lipase is typical of those causing in vivo fat digestion, it can be reasoned that the chemical identities of free fatty acids, 2-monoglycerides, and 1,2-diglycerides that result will be determined by the positioning of the fatty acids in the glycerides of the ingested fat. Since it is probable that these three types of end products enter somewhat different metabolic pathways in the body, one might expect the glyceride structure of a dietary fat to be of more than academic interest.[4] At least it should be of practical interest in the dairy field, because milk fat seems to have a triglyceride structure at variance with those of many other edible fats (3-5).

The technical assistance of T. R. Lynn in the experimental work reported is gratefully acknowledged.

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[4] In fact, the triglyceride structure of animal fats may, in part, simply reflect the structure of ingested fats, because of the manner in which lipase acts.

THE ORIGIN OF DEPOT FAT[1, 2]

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In the past, students of nutrition and biochemistry were taught that the fatty acid composition of an animal's fat lay somewhere between that synthesized from carbohydrate (but characteristic of the species) and that of the fatty acid composition of the dietary fat. Associated with this was an over-all concept of randomness. The depot fat of the animal was presumed to contain a random assortment of the fatty acids synthesized from carbohydrate and those absorbed from the diet. Ideas on the fatty acid make-up of a triglyceride molecule underwent a metamorphosis, largely because of the work of Hilditch and his associates, from concepts that a fat was a mixture of simple triglycerides (e.g., triolein, tripalmitin, tristearin, etc.) to the concept that fats were mixtures of mixed triglycerides, i.e., glyce-

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erides containing different fatty acids (12). However, it was generally assumed that an acid could occupy with equal probability any of the three positions on the glycerol molecule. Recently, the development of new techniques has revealed that these ideas are not entirely true and are an oversimplification of the actual picture.

Previous notions also held that animal fats were composed of saturated acids containing an even number of carbon atoms occurring in straight chains. The unsaturated acids were held to be similarly constructed with double bonds in a *cis*-configuration only.

The presence of fatty acids with odd numbers of carbon atoms were first shown to occur in ruminant fats (9), but more recently the high resolving power of gas chromatography has shown that the fats of all animals probably include small amounts of fatty acids containing an odd number of carbon atoms and/or branched chains (16, 18, 19). Moreover, by means of infrared spectroscopy, unsaturated fatty acids with transdouble bonds have been found in ruminant fats (10). Although animal fats may contain more than 35 fatty acids (16, 18) six usually comprise more than 90% of the fatty acid composition of animal depot fats. These are myristic acid (14:0), palmitic acid (16:0), and stearic acid (18:0) of the saturated acid series, and palmitoleic (16:1), oleic (18:1), and linoleic (18:2) of the unsaturated series. All of these acids, except linoleic acid which can only arise from dietary fat, can be synthesized by the animal from carbohydrate or other fatty acids.

The synthesis of triglycerides from fatty acids occurs by the mechanism shown in Figure 1. The fatty acids are first activated to the coenzyme-A esters by ATP (20). Two molecules of fatty acyl–CoA condense with L-α-glycerolphosphate to form a phosphatidic acid (21) which is converted to an α-β-diglyceride by action of a specific phosphatase (36). The α-β-diglyceride is then esterified by a third fatty acyl–CoA forming the triglyceride (42).

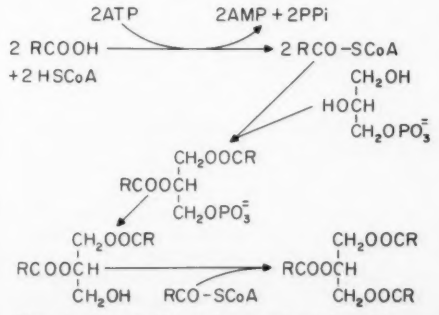

FIG. 1. Metabolic pathway of triglyceride synthesis.

DEPOSITION OF DIETARY FATTY ACIDS

Nonruminants. The concept that a dietary fatty acid is deposited in the depot fat of an animal rests largely upon the work of Ellis and co-workers (2) and their efforts to solve the soft pork problem. This concept was further borne out by other early work with nonruminant animals (2). The relative constancy of fatty acid composition of ruminant fat resulted in the theory (34) that the dietary fat of ruminants was not incorporated but that the fatty acids were broken down by the rumen bacteria.

Most of these investigations depended on analytical methods, such as iodine number and saponification number, which yield only mean values of such parameters as unsaturation and chain length. Later techniques such as alkaline isomerization and fractional distillation resulted in improved analyses but still lacked fatty acid specificity, or were time-consuming and required large samples of fat. Despite these difficulties, it was apparent that dietary caproic acid (5), lauric acid (27), and myristic acid (4) were deposited in the depot fats of nonruminant animals. Among the unsaturated acids, oleic acid (4), linoleic acid (37), and other unsaturated acids having a chain length of C-18 or greater were also deposited in body fat when fed.

The fate of dietary palmitic acid and dietary stearic acid was still unknown and the effect of dietary level of a particular acid on the deposition of that acid was largely unexplored. The advent of gas chromatography, permitting the analysis of fatty acid composition of a minute sample of fat in a matter of minutes, enabled the investigation of these facets of the problem.

The results of studies on the effect of feeding graded levels of oleate, linoleate, palmitate, stearate, and myristate on the deposition of these acids in the fat of mice show very little similarity between the acids in their deposition pattern. The oleic acid content of the depot fat increased with the dietary oleate until a level of 67% was attained and thereafter remained constant, even though the dietary oleate increased more than twofold (40). Myristic acid behaved in a similar fashion, except that a maximum level in the depot fat was reached at a 40% level (43). The curve representing the deposition of dietary linoleate showed an initial rapid deposition followed by a slower deposition, with a change in slope occurring when the level of linoleate in the depot fat reached 45% (40). Contrary to the aforementioned acids, very little palmitate and stearate were deposited, even though the diet contained as much as 50% of the monoglycerides of these acids (41).

The lack of deposition can not be ascribed to lack of absorption and, hence, one is left with the conclusion that dietary palmitate and

stearate are more readily oxidized than the other dietary acids. Perhaps related is the observation that high levels of saturated fat are toxic to monogastric animals (1, 11, 41). The addition of small amounts of linoleic acid, oleic acid, or any unsaturated fat prevents this toxicity, but the mechansim of action is not understood.

Ruminants, depot fat. The influence of dietary fat on the fat of ruminants has been almost entirely confined to studies on milk fat. The most striking factor apparent in all of these studies is the relative inertness of the fatty acid composition of the depot fat of these animals to change by dietary fat (14). It is becoming increasingly apparent that the inertness is not so much the result of complete degradation of ingested fatty acids by the rumen microorganisms but rather is the result of hydrogenation of unsaturated fatty acids, particularly the C-18 acids. The hydrogenating action of the rumen microflora was first shown by Reiser (28) and later confirmed by others (17, 35). Furthermore, the presence of trans-unsaturated acids, occurring in more than trace amounts only in the fat of ruminants (10), has been shown to occur as a result of the action of rumen microflora (35). It is possible that the process of hydrogenation and the production of transacids by rumen microflora may be closely related. For example, the rumen microflora could contain a reversible hydrogenation-dehydrogenation system that could produce a transacid from the hydrogenated (or partially hydrogenated) derivative of a *cis*-acid.

Edwards *et al.* (7), using gas chromatography, studied the changes in the fatty acid composition of the depot fat of steers when fat was added to the diet of these animals. Only stearic acid was found to increase, despite the fact that added fat was rich in oleate and linoleate (Table 1). Similar observations were made by Mochrie and Tove on brisket fat of Holstein cows fed whole soybeans (25).

TABLE 1

Dietary fat level and fatty acid composition of beef tallow (6)

Fatty acid	Per cent dietary fat		
	2.0	5.8	7.9
	Per cent tallow fatty acid		
18:0	14.5	16.8	21.4
18:1	42.2	41.1	40.0
18:2	1.2	0.7	0.3

Ruminants, milk fat. In comparison to the depot fat of a ruminant, the fatty acid composition of milk fat from the same animal is considerably more changeable. The well established difference (14) between summer and winter butter serves as an illustration of this point. Certainly, the greater effect of dietary fat on the fatty acid composition of milk fat compared to depot fat should not be surprising, for the entire milk fat is synthesized in a few hours, whereas in the fat depots newly synthesized fat is diluted by pre-existing fat stores.

Several investigators (3) have shown that adding an unsaturated fat to the diet of cows results in an increase in the iodine number of the milk fat. The incorporation of dietary fatty acids into milk fat is shown by the fact that the addition of 0.5 lb. of peanut oil to the diets of cows resulted in an increase in the oleic acid level of milk fat, and that feeding laurie and myristic acids in the form of palm oil or coconut cake increased the content of these acids in the milk (14). Also, feeding cod liver oil and rape seed oil resulted in milk fat containing small amounts of the unsaturated C_{22} acids that are found in these oils (14).

It should be emphasized that the fatty acid composition of milk fat is more apt to reflect the fatty acid composition of a dietary fat when added to the diet as a free oil than when furnished as a natural product, or in the natural form. For with the free oil there is less chance for microbial action to change the fatty acids than with the slower release of the acids from a natural product. The addition of a free oil further complicates the problem by altering the microbial population (33) and, concomitantly, the amount of fat in the milk.

To circumvent these problems and to study the influence of dietary fat on milk fat composition, Mochrie and Tove (25) studied the effects of dietary soybean oil, fed as the whole bean, on the fatty acid composition of milk of Holstein cows. Typical results (Table 2) show that the levels of both stearate and oleate increase with soybean feeding and immediately return when the soybeans are removed from the diet. In contrast, linoleic acid increased only slightly with soybean ingestion. These results coupled with those of others (14) show that the oleic acid content of milk is more easily influenced by diet than is the level of linoleic acid. In contrast to the results of feeding sources of linoleic acid, recent investigations by Mochrie and Tove (25) have shown

TABLE 2

Fatty acid composition of milk from cows fed soybeans (25)

Fatty acid	Weeks [a]		
	2	10	12
	(%)		
14:0	11.5	6.0	13.0
16:0	30.0	24.2	32.8
16:1	4.1	3.3	2.9
18:0	13.0	26.4	15.4
18:1	29.9	35.8	26.0
18:2	2.1	3.2	2.0

[a] For the first 2 wk. the cows were fed the herd diet; whole soybeans were fed from Weeks 3 to 11; after Week 11 the herd diet was fed.

that the intravenous infusion of cottonseed oil substantially increases the level of linoleic acid in milk fat (Figure 2).

These results dramatically demonstrate that, even though relatively large quantities of linoleic acid are ingested, the effective hydrogenation of dietary linoleic acid by the rumen microorganisms precludes the occurrence of a high level of this fatty acid in the milk and depot fat of ruminants. For once the rumen is by-passed the cow, like a monogastric animal, readily incorporates linoleic acid into milk fat.

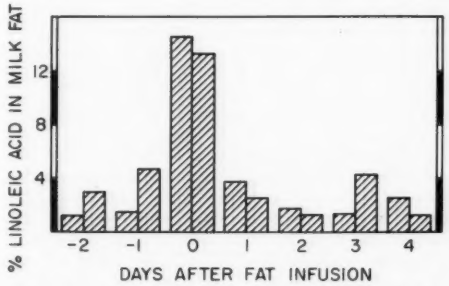

FIG. 2. Effect of intravenous infusion of cottonseed oil on the linoleic acid content of milk fat. A Holstein cow was given 450 g. of cottonseed oil (Lipomul kindly furnished by Upjohn Company, Kalamazoo, Michigan) over a 2-hr. period. The double bars for each day represent evening and morning milk, respectively.

INFLUENCE OF ONE FATTY ACID ON THE FATTY ACID DISTRIBUTION IN DEPOT FAT

Although there were early observations of change in the fatty acid distribution of animal depot fats that could not be accounted for by the fatty acid composition of the dietary fat, the reasons behind these changes were not explored. Some examples of these changes are:

1. Increased level of stearic acid in the depot fat of rats fed cottonseed oil (8, 37) which contains only 1% stearate.
2. A decreased level of palmitate in depot fat, even when animals were fed fats containing more than 20% palmitic acid (13).
3. A decreased level of palmitoleic acid in depot fats as the amount of dietary fat increased (13).
4. A constant level of unsaturated acids, even though the level of linoleic acid varied widely (29, 38).

Clearly, investigations of the nature of these fatty acid changes would necessitate a large number of fatty acid analyses that if carried out by distillation of the fatty acid–methyl esters would be a formidable task indeed. However, the development of the gas chromatography technique enabled the conduction of such an investigation.

Using this technique we have investigated the influence of one fatty acid on the distribution of the other fatty acids in the depot fat of mice. In all cases, various elevated levels of a given fatty acid in the depot fat were produced by dietary means.

To remove any effects caused by mere dilution of the depot fat by the acid being tested, the relative per cents (RP) of the various fatty acids were computed. The RP is merely the per cent of a given fatty acid divided by [100 minus the per cent of the dietary acid in the depot fat]. The changes in RP that resulted from these investigations are summarized in Table 3.

TABLE 3

Changes in the fatty acid composition of mouse depot fat

ACID FED	CHANGES IN DEPOT FAT					
	14:0	16:1	18:2	16:0	18:1	18:0
18:2(A)	—	↓	↑	—	—	↑
18:2(B)	↓	↓	↑	↓	—	↑
18:1	↑	—		↓	↑	?
14:0	↑	↓		↓	—	—
16:0	—	—		↑	↓	—
18:0	—	↓		—	↑	↑

As the linoleic acid content of the depot fat increased to a level of about 45% (18:2A, Table 3) the RP of palmitoleic acid decreased and the RP of stearic acid increased (40). No statistically significant ($P < .05$) change was observed for the RP of myristic acid or the RP of oleic acid. In some experiments the RP of palmitic acid increased, but in others no statistically significant trend was noted (40). When the level of linoleic acid in the depot fat exceeded the 45% level (18:2B, Table 3), statistically significant changes ($P > .01$) were found for all acids except oleic acid. An increase in linoleate was accompanied by an increase in the RP of stearic acid, but a decrease in RP of myristic acid, RP of palmitic acid, and RP of palmitoleic acid (40). The effect of linoleate on the increase in stearic acid is dramatically indicated by the fact that the absolute per cent of stearic acid in the depot fat of mice fed safflower oil (74% linoleic acid) is greater than that for mice fed a comparable level of glycerol-monostearate (41).

With oleic acid, an entirely different pattern was observed (40). An increase in the level of oleic acid in the depot fat was accompanied by a decrease in the RP of palmitic acid and an increase in the RP of myristic acid, whereas palmitoleic acid showed only random changes. Alterations in the level of stearic acid were

indeterminate because of a C-17 unsaturated acid present, as a contaminant, in the dietary fats that interfered with the determination of stearic acid.

When trimyristin was fed, the RP of palmitic acid and palmitoleic acid in the depot fat decreased, but no change was observed in the RP of oleate or the RP of stearate (43). An increase in the level of palmitic acid in the depot fat was accompanied by a decrease in the relative level of oleic acid only (41). Although the addition of glycerol-monostearate to the diet raised the level of stearic acid in the depot fat only slightly, there was a decrease in the palmitoleic acid level of the depot fat and an increase in the level of oleic acid (41).

It will be noted (Table 3) that in each case the dietary fatty acid exerts a specific effect on the relative distribution of the other fatty acids in the fat. Furthermore, each dietary fatty acid is associated with a different change in the relative distribution of the other fatty acids of the depot fat. The mechanism whereby one fatty acid exerts a specific influence on the deposition of others in the depot fat is not known. However, the high degree of specificity in the patterns of change of the fatty acids would strongly suggest that the mechanism for this lies in the specificity of the enzymes associated with fat metabolism. In speculating on the possible metabolic systems that could be involved, it would seem reasonable to suspect either one or both of the enzyme systems associated with the esterification of glycerol (Figure 1). The specific interactions among the fatty acids could also be accounted for by lipases, having high degrees of substrate specificity, catalyzing the exchange of one fatty acid for another.

The solubility properties of fatty acids are such that the presence of a double bond is equivalent to removing two carbon atoms; e.g., myristic acid, palmitoleic acid, and linoleic acid appear together in the eluant of liquid-liquid chromatography systems (24). Mead (24) had observed an increased level of palmitoleic acid in the fat of rats deficient in linoleic acid, and suggested that the similar physical properties of these two acids might be involved in the replacement of one for the other. The acids of Table 3 are arranged in groups according to their solubility properties. It is noteworthy that in every case, except stearic acid, the elevation of an acid by dietary means is accompanied by a decrease in an acid of the same solubility class. Thus, these observations are consistent with the suggestion of Mead (24) and would suggest that the metabolic machinery of an animal is so constructed as to maintain a homeostasis of the physical properties of the fat.

DISTRIBUTION OF FATTY ACIDS ON THE GLYCERIDE STRUCTURE

When natural fats were found to be mixtures of molecules containing different fatty acids, rather than simple triglycerides, the question arose whether the esterification of a given fatty acid to a particular glycerol hydroxyl group was selective or random. Studies on isolated triglycerides of natural fats by means of X-ray diffraction spectra and melting point had uncovered examples of nonrandom configuration, but these studies were too few in number to permit any generalizations (15). Fortunately, a more suitable tool for such a study was found in pancreatic lipase. This enzyme was shown by Savary and Desnuelle (30, 31) and Mattson and Beck (22) to preferentially hydrolyze the fatty acids esterified to the α-hydroxyl groups of glycerol. Thus, by examining the isolated products of the hydrolytic action of this enzyme, the structure of natural triglycerides could be studied.

Using pancreatic lipase, triglycerides from both plants and animals have been shown to have a nonrandom configuration (23, 32). In general, in both plant and animal triglycerides the β position of the glycerol is predominantly esterified by an unsaturated acid, whereas the saturated acids predominantly occupy the α positions (Table 4). A notable exception to this generalization is pig fat, in which the structure is reversed (Table 4). Here, the majority of the saturated acids are found in the β position and the unsaturated acids predominate in the outer position of the glyceride. Milk fat tends to resemble pig fat, but the disparity of distribution is not as wide (26).

TABLE 4
Distribution of fatty acids in animal triglycerides (22)

Source	Per cent of acid in β		
	Saturated	Monoenoic	Dienoic
Beef	18	49	88
Horse	18	31	69
Sheep	19	51	68
Man	19	38	61
Dog	28	31	58
Rat	39	22	78
Pig	65	16	17

Studies of the effect of a dietary fatty acid on the fatty acid distribution of depot fat were extended to an investigation of changes in the configuration of the triglycerides of the depot fat by means of the pancreatic lipase technique (23). Further impetus for this extension was the possibility that the two patterns of change in the fatty acid distribution, observed with linoleate feeding (Table 3), might be associated with different positions in the triglycerides.

The results of such a study (39) show that the distribution of this acid differs with the amount present (Figure 3). For, although the β position initially contained over half the

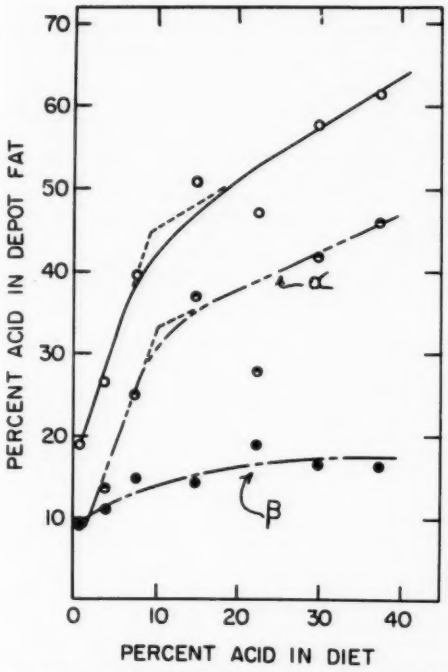

FIG. 3. Effect of dietary level of linoleate on the distribuation of linoleic acid in depot fat. ○ linoleic content of total fat, ◓ linoleic acid content of the two α positions of the triglycerides, ● linoleic acid content of the β position of the triglycerides.

linoleic acid, the predominant increase in linoleic acid occurred in the α position. The change in the rate of linoleate deposition occurring when the α positions are half-saturated (33% with a maximum of 67%) provides a basis for the interesting speculation that the two slopes might represent the two α positions, or that the presence of a linoleic acid molecule in one α position decreases the degree of esterification of the other α position by that acid. Preliminary results (41) indicate that an increase in the linoleic acid level of the β position does not induce a change in the relative distribution of the other fatty acids, and that the two patterns of change observed with the whole fat (Table 3) are associated with the two slopes of the α-esterified linoleic acid.

SUMMARY

Largely because of the recent development of analytical techniques, many of the older notions concerning the depot fat of animals are undergoing revision. Although any dietary fatty acid of chain length greater than ten carbon atoms will be deposited in the body fat of a nonruminant animal, the rate and extent of deposition will vary widely, depending on the fatty acid in question. With ruminant animals the capacity of the rumen microflora to hydrogenate unsaturated acids plays the dominant role resulting in the deposition of saturated fatty acids. The fatty acid composition of animal fats results from highly specific and controlled metabolic reactions. This specificity concerns not only the influence of one fatty acid on the distribution of the others but also the position of the fatty acids on the glycerol molecule.

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AUTOXIDATION OF MILK LIPIDS[1]

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Milk lipids are recognized as a major contributor in determining consumer acceptability of most dairy products. However, the reaction of these lipids with oxygen, resulting in flavor deterioration, creates serious problems in storage stability. Quality impairment due to autoxidation is widespread and manifests itself in many ways, depending upon the product involved. In certain products, particularly dry whole milk, autoxidation has been a major deterrent to the development of an acceptable product. In all products, considerable cost is added to provide protection against oxidative effects. Whatever is done to milk fat to improve the appeal of dairy products, whether it be by alteration of its physical properties or by more attractive prices through efficient production, we must be cognizant of the need

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to provide maximum storage stability. Current trends in marketing make this requirement more essential than ever.

No other chemical deterioration has been studied as extensively as autoxidation, nor has any other problem been investigated from so many different angles. Even so, controversy still prevails relative to the origin of the flavor compounds, the mechanism of the reactions, and the influence of environmental factors on the nature of the oxidation products. In a field as large and as controversial as autoxidation, brief treatment as desired here only can include some of the current thinking, with supporting evidence wherever possible. More thorough treatments on the autoxidation of dairy products are included in reviews by Brown and Thurston (2), Greenbank (13), and Pont (24), and on lipids in general by Morris (19), Holman (15), and Lea (17).

IDENTITY OF COMPOUNDS IN OXIDIZED MILK LIPIDS

One of the most fruitful approaches to the study of lipid oxidation deals with elucidation of hyproperoxide degradation products. Until recently, progress was hindered by lack of adequate tools to attack the problem. However, developments in chromatography and spectrophotometry over the past decade have provided many of the necessary implements and, as a result, the identification of compounds responsible for oxidized flavors is now being investigated in many laboratories. Identification of the flavor compounds will be valuable in revealing differences in the sensory character of the flavor defects, in providing the necessary information for a direct chemical measurement of the flavor and in elucidating the reaction mechanisms of lipid oxidation which are by no means clear at the present time.

One of the first serious attempts to identify the flavor of oxidized milk lipids was made during World War II by van der Waarden (28) on cold storage butter. He observed that the flavor compounds were fat soluble and could be completely removed from the fat by degassing at a low pressure. The volatile compounds, isolated by this technique, were low in nitrogen, sulfur, and phosphorus, contained no peroxides, and imparted an oxidized flavor when added to fresh fat. From micro-qualitative studies of the volatiles and upon comparison of properties of the distillate with pure compounds, it was postulated that methyl ketones and aliphatic, unsaturated, unconjugated aldehydes were of importance to the flavor. Two other reports (16, 27) in the literature largely support the findings of van der Waarden and are the basis for the current belief, by many investigators, that unsaturated, unconjugated carbonyls are responsible for the oxidized flavor of fat. Keeney and Doan (16), in a study of oxidized fat, concluded that the bulk of the odor compounds was ketones. None of the ketones was methyl ketone and a large portion was unsaturated. Their conclusions were based upon the specificity of Girard's reagent for fractionation of aldehydes and ketones. The Girard's reagent has since been shown to be nonspecific (8) and it is likely that the compounds isolated by Keeney and Doan (16) were saturated and α,β-unsaturated aldehydes. Evidence for ketones in their carbonyl mixtures is not surprising in view of the heat treatment given the fat and the recent findings by Patton and Tharp (22) that ketones are produced by heat treatment.

Tamsma (27) also studied the volatile compounds from milk fat and reported on three classes of carbonyls contained in the Skellysolve soluble fraction. The unconjugated, unsaturated compounds were reported to be responsible for the flavor and along with the conjugated monene compounds, constituted the major portion of the volatile fraction. Conjugated dienes were present at trace levels and were reported to be ketonic. Tamsma's conclusions were based largely on hydrogenation studies, in which it was claimed that the conditions of hydrogenation preferentially saturated double bonds without reducing the carbonyl groups. There was no direct evidence given to substantiate this claim. In fact, critical examination of the data would infer that the carbonyl group was reduced. Based on hydrogen uptake, Tamsma found the molar absorbance of the conjugated diene fraction to be 10,400 at 265 mμ, yet values in the literature are in the range of 26,000 and above (10, 23). This would suggest that the amount of hydrogen absorbed was substantially more than was necessary to saturate the double bonds. Therefore, an alternate conclusion is that hydrogenation eliminated oxidized flavor by reduction of the carbonyl group.

Unfortunately, refined chromatographic procedures were not available at the time the aforementioned studies were conducted. A careful examination of the data shows similarities to more recent findings. For instance, the fraction termed unsaturated, unconjugated by Tamsma appears similar to the saturated aldehydes found by Day and Lillard (5) and by Forss *et al.* (9). Similarly, the conjugated monene and diene compounds appear similar to 2-enals and 2,4-dienals (5, 9, 11).

Forss, Pont, and Stark (11) conducted a series of experiments in which they isolated and identified the compounds causing the copper-induced oxidized flavor of skimmilk. Ethanal, n-hexanal, the C_4 to C_{11} alk-2-enals, and the C_5 to C_{11} alk-2,4-dienals were identified. The most abundant compounds were the C_8 and C_9 2-enals and the C_7 and C_9 dienals. These compounds were isolated from 100 gal. of oxidized skimmilk. This quantity of skimmilk would contain approximately 75 g. of phospholipid (0.02%) and about 200 g. of fat (0.05%). Although it was not specified in

their reports, phospholipid oxidation probably was responsible for a large proportion of the compounds.

van Duin (7) has studied the products of butter phospholipids when oxidized at pH 4.6 and 13° C. He presented tentative evidence for the C_2 to C_{18} alkanals, the C_5 to C_{11} alk-2-enals, the C_8 and C_9 alk-2,4-dienals, and small quantities of a number of different ketones. He subsequently found that the longer chain carbonyls, $> C_{12}$, were present in the phospholipids as acetals (plasmalogens) and were not products of oxidation. The flavors of the long chain aldehydes were described as pleasant and it is unlikely that they contribute to oxidized flavors. Their possible significance in natural flavors warrants further investigation.

Day and Lillard (5) identified the C_1 to C_{10} alkanals, the C_4 to C_{11} alk-2-enals, and obtained evidence for the odd-numbered C_3 to C_{15} methyl ketones in oxidized milk fat. The ketones were not attributed to oxidation since they were found in fresh fat. Since the above work was published, we have also found evidence for traces of hepta-2,4-dienal in the fat. This was accomplished by refinement of our techniques through the use of the paper chromatography method of Gaddis and Ellis (12) in combination with column partition chromatography (4).

In a recent work, Forss *et al.* (9) have identified a number of the compounds that are responsible for the fishy flavor in butterfat. They employed a combination of procedures which included gas, paper and column chromatography. Two fractions with distinct flavors were obtained. One fraction exhibited an oily flavor and contained n-hexanal, n-heptanal, and 2-hexenal. The other flavor fraction was described as metallic and appeared to contain a single carbonyl compound in relatively small amounts. Compounds that were identified included the C_3 and C_5 to C_{10} alkanals, the C_3 and C_5 to C_9 alk-2-enals, hepta-2,4-dienal, a compound resembling octa-2,4-dienal, a compound with a mushroom flavor, and a compound that formed the 2,4-dinitrophenylosazone of methylglyoxal. It is interesting that most of the compounds found by Forss in fishy flavored fat were also isolated from typical oxidized milk fat by Day and Lillard (5). Day and Lillard (5) found the complete series of alkanals from C_1 to C_{10} and the C_4 to C_{11} alk-2-enals whereas Forss *et al.* (9) found the C_3 and not the C_4 enal and they did not find the C_1, C_2, and C_4 alkanals nor the C_{10} and C_{11} alk-2-enals. Also, Forss *et al.* (9) found the unidentified metallic and mushroom fractions. These unidentified fractions could have been missed by our techniques since they may occur in the dicarbonyl fraction. Likewise, the C_{10} and C_{11} enals could have been missed by Forss *et al.* (9), due to temperature limitations of their gas chromatography method.

At the present time, it appears that the majority of the volatile monocarbonyl compounds occurring in oxidized milk lipids belong to three classes; namely, the alkanals, alk-2-enals, and alk-2,4-dienals. There are probably traces of other compounds and these uncommon ones may well have an influence on oxidized flavor. However, recent studies under different conditions of oxidation show that practically all of the monocarbonyls will fit into the above classes (5, 9). The generally accepted free radical mechanism of lipid oxidation will explain the formation of most of the carbonyls, provided the unsaturated fatty ester precursors are present in the system.

STUDIES ON THE SENSORY CHARACTER OF OXIDIZED FLAVOR

Since the volatile monocarbonyls are largely responsible for oxidized flavors of dairy products, qualitative and quantitative variations of the flavor mixture must account for differences in flavor character. For example, qualitative differences appear responsible for the specific flavors of oxidized skimmilk as compared to those of milk fat. Alk-2-enals and alk-2,4-dienals predominated in skimmilk (11); whereas, alkanals and alk-2-enals are the major flavor compounds in oxidized milk fat (5). Differences in the qualitative composition of oxidized flavors are influenced by the composition and susceptibility of the substrate. The phospholipids of fluid milk contain a higher percentage of polyethenoic acids than do the triglycerides (26). Furthermore, the phospholipids being oriented at the fat-serum interface are conceivably more susceptible to oxidative attack. Thus, in fluid milk it is believed that the phospholipids are preferentially oxidized. There is a wealth of circumstantial evidence to support this view. It is questionable, however, if the flavor comes entirely from phospholipid oxidation. The intimate association of phospholipids and triglycerides at the fat-serum interface in milk should afford conditions for oxidation of both. It is unlikely, therefore, that the triglycerides are completely free from oxidative attack, especially in the presence of the peroxides and the free radicals resulting from phospholipid oxidation. Oxidation of the more susceptible and highly reactive phospholipids would predominate, nevertheless.

The influence of quantitative changes in the volatile carbonyl mixture on the flavor character of oxidized fats has been largely ignored in the past. This problem is of importance when attempting to relate objective to sensory methods in flavor studies. If the percentage composition of the carbonyl mixture varies, correlation of individual compounds with flavor intensity may be impractical, unless the flavor is due to a single compound. However, if the percentage composition remains constant regardless of flavor intensity, a single compound should give high correlation with sensory measurements. In a study of 16 milk fat

samples covering a wide range of oxidation (peroxide values 0 to 64), Day and Lillard (6) observed considerable variation in the percentage composition of the volatile monocarbonyl mixtures. Certain carbonyls always predominated but their percentage of the mixture was not constant. Furthermore, the carbonyls at highest concentration did not give the highest correlation with oxidized flavor intensity. The mean percentage of heptanal for 16 samples was 18.8% but its correlation with flavor intensity was 0.426. In contrast, the mean percentage of non-2-enal was 4.18% but its correlation with flavor was .942. It is significant that Holm *et al.* (14) also found a high correlation between the α,β-unsaturated carbonyls and the oily flavor of butters.

The oxidized flavor intensities of the 16 milk fats were ascertained by determining the absolute flavor threshold (FT) of the fat in whole milk media. By knowing the FT of the oxidized fat and the amount of each monocarbonyl in the fat, it was possible to calculate the concentration of carbonyl compounds required to give a perceptible oxidized flavor in whole milk. It was found that at the FT of the fat, the carbonyls were present in concentrations of parts per billion. In most cases, the individual compounds were at marginal or subthreshold levels. This was based on published flavor thresholds for carbonyl compounds (18). There is insufficient sensory data to draw conclusions but it appears likely that when the oxidized flavor of milk is just perceptible, the flavor may be due to the additive effects of compounds, with each compound occurring at subthreshold levels, but the combined concentrations equaling or exceeding the threshold of any one in the mixture. Compounds such as the C_7, C_8, and C_9 enals, which are very similar in sensory character, may act in such a way.

The same phenomenon may explain observed differences in flavors of oxidizing milk fats. A case in point is the tallowy (5) and fishy (9) flavors of oxidized milk fats. It is notable that the volatile monocarbonyls from both fats are practically identical. The most striking difference in the data from the two fats is the percentage composition of the carbonyl mixtures. A comparison of the data is given in Table 1.

TABLE 1

Per cent composition of carbonyls from tallowy and fishy flavored milk fats

Carbonyl	Tallowy[a]	Fishy[b]	Carbonyl	Tallowy[a]	Fishy[b]
C_1+C_3al	32.47	....	C_8enal	.....	?
C_3al	5.04	?	C_4enal	0.42	....
C_4al	1.41	....	C_5enal	1.46	3
C_6al	9.96	4	C_7dienal		8
C_8al	9.23	25	C_8enal	0.38	4
C_7al	18.80	20	C_7enal	1.91	3
C_8al	3.28	1	C_6enal	0.66	2
C_9al	2.70	5	C_9enal	4.18	5
C_{10}al	1.82	1	C_{10}enal	1.23	....
C_{13}one	4.01	....	C_{11}enal	1.08	....

[a] Mean of 16 samples (6).
[b] Taken from Forss *et al.* (9).

STUDIES OF DICARBONYL COMPOUNDS IN OXIDIZING MILK LIPIDS

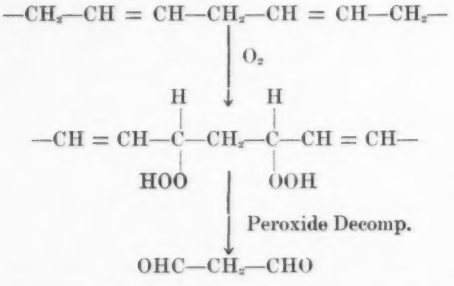
The dicarbonyl compounds of oxidized milk lipids have received limited attention in the studies of lipid oxidation. Most investigators have eliminated these compounds as possible contributors to flavor defects but this is open to question. These compounds comprise a significant portion of the volatiles from oxidized fats and even though they may not contribute directly to the flavor, their reactivity in browning type reaction makes them worthy of consideration. Such reactions undoubtedly occur in oxidizing dairy products where proteins are present. Therefore, possibilities for both flavor and physical defects in dairy products through lipid browning reactions deserve consideration.

The main reason that dicarbonyls have not been studied more extensively is the difficulty in handling them, either in the free or derivative form. The most common reagent used is 2,4-dinitrophenylhydrazine and mixtures of the derivatives are troublesome to separate because of solubility problems. The use of gas chromatography for separating the free compounds, and the development of suitable paper and column chromatography procedures for the derivatives, will be valuable adjuncts in elucidating the nature of these compounds. Forss (9) has found evidence for a C_9-α-dicarbonyl in oxidized fat by gas chromatography and we have recently fractionated the dicarbonyl mixture of oxidized milk fat into six fractions. The identity of these fractions has not been determined and the mixture obviously is complex.

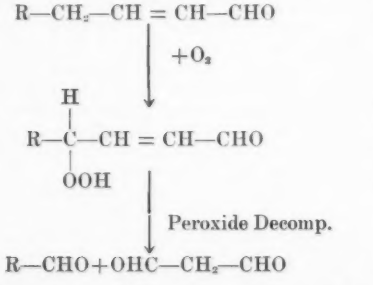
Most of the work on dicarbonyls has been concerned with the nature of the reactants in the Kreis and TBA tests. Patton and Kurtz (20) obtained evidence that malonaldehyde was the compound responsible for the reaction in oxidized fats. More recently, Sinnhuber *et al.* (25) isolated a TBA pigment from oxidized salmon oil which had properties identical to the TBA pigment of malonaldehyde. The unknown TBA pigment was homogenous and they concluded that malonaldehyde was the compound responsible for the TBA reaction.

The origin of malonaldehyde in oxidizing lipids poses some interesting questions relative to the mechanism of the oxidation reaction. Bernheim *et al.* (1) reported that oxidized oleic acid gave a negative TBA test, linoleic acid only a slight reaction; whereas, linolenic acid gave a strong test. Patton and Kurtz (21)

have reported that oxidized 2-enals give the reaction. An explanation of how malonaldehyde can be formed through generally accepted oxidation schemes is still forthcoming. One can only speculate on possible mechanisms. In polyethenoic acids, containing the pentadiene system, diperoxide formation at the double bonds, with a simultaneous shift in double bonds, followed by cleavage of the peroxides could give malonaldehyde.



Diperoxides have not been reported as initial oxidation products in unconjugated fatty acids and since TBA reactants are detected at very early stages of oxidation the above scheme is questionable. In α,β-unsaturated aldehydes, the carbon alpha to the double bond is labile and may be susceptible to oxidative attack. If so, malonaldehyde could arise through the following mechanism.



Quantitative studies of the TBA reactant in fats have shown that the only part of the malonaldehyde exists free in the fat. Patton and Kurtz (20) could remove only part of it by water extraction. Sinnhuber *et al.* (25) removed 2.2% by nitrogen ebullition of salmon oil and Day and Lillard (6) have been able to remove approximately 10% by vacuum steam distillation. Consequently, it can be assumed that most of the malonaldehyde is produced during the reaction by breakdown of a precursor, by dissociation of dimers, or by other mechanisms. It is of particular interest that in running the TBA test, conditions are conducive for peroxide decomposition. It is also notable that Day and Lillard (6) have found the TBA and peroxide tests to be highly correlated, .962. While evidence is still inadequate to justify conclusions, it would appear that the TBA test is an indirect measure of the free malonaldehyde plus the peroxides when used on intact fats. A similar situation may not hold for foods such as whole milk. Peroxide decomposition may enable the highly reactive malonaldehyde to combine with available amino groups to give a Schiff's base. The conditions employed in the TBA test could reverse the reaction and enable measurement of the bound malonaldehyde, provided an Amadori rearrangement has not occurred.

AREAS FOR CONTINUING RESEARCH

If nothing else, this presentation should indicate the need for more research on lipid oxidation. The oxidation reaction in dairy products is very complex and it is influenced by a multitude of factors. The identification of lipid peroxide decomposition products is far from complete. The effect of metals, light, antioxidants, vitamins, and other factors on the types of oxidation products formed needs more thorough study. In addition, the reaction of secondary products of lipid oxidation with other constituents in dairy products deserves consideration. Browning reactions involving products of lipid oxidation have been reported in dried eggs (17). The reaction appears similar to heat induced browning except that the carbonyl apparently is derived through lipid oxidation. This type of reaction has been largely ignored in dairy products, but it is conceivable that it may be of significance in flavor deterioration.

There is still controversy on the subject of spontaneous oxidation in fluid milks. Whether the cause of the reaction is enzymatic, metal catalyzed, or both is currently controversial. In any case, it is known that the ration of the dairy cow has some influence on the incidence of oxidized flavors in the milk. The incidence of spontaneous oxidation is highest when the animals are on hay and concentrated rations. The lipids in hay and concentrates should oxidize quite readily. Consequently, it is conceivable that dry feeds could contain appreciable amounts of lipid peroxides. The question arises: Is it possible for lipid peroxides of the ration to be consumed by the cow and synthesized into milk lipids? The low Eh of the rumen would be conducive to peroxide reduction, but is the rumen 100% efficient? Apparently, it is not in the case of long chain unsaturated fatty acid synthesis. If this possibility is ruled out, is it possible for lipid peroxides to form in the fatty tissue of the cow and subsequently mobilize for milk fat synthesis? Peroxides have been detected in the depot fat of chickens (3) and swine and rats (17) where their diets contained either oxidized fats or unsaturated fatty acids and were complicated by lack of vitamin E. Theoreti-

cally, it would take only one hydroperoxide in the milk lipids to initiate the oxidation reaction. If there is a remote possibility that lipid peroxides can be synthesized, their effect on spontaneous oxidation of fluid milk is obvious.

Many terms have been used by different workers to describe oxidized flavors in dairy products. Such terms as stale, tallowy, oily, metallic, fishy, and cardboard are quite common. Many of these terms may be justified, but it makes communication among investigators practically impossible. Standardization of flavor terminology is urgently needed. Rather than describe a flavor as tallowy it would be more desirable to associate it with a specific compound. Continued research on the identification and the sensory evaluation of compounds responsible for oxidized flavors should give the necessary reference compounds for this purpose.

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INTERPRETIVE SUMMARIES OF PAPERS

STRUCTURE AND SYNTHESIS OF MILK FAT. I. GAS CHROMATOGRAPHIC ANALYSIS

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Milk fat is one of the most important dietary fats. It is also important in the flavor, physical properties, and keeping quality of most milk products. The particular bearing a given sample of milk fat may have on these considerations will depend upon its composition. One of the most meaningful analyses that can be made of fat composition is in terms of component fatty acids. If milk fat is to be developed or selected for particular use, because of special physical, chemical, or nutritional requirements of milk products, this will be accomplished through variations in fatty acid composition.

A convenient method of analyzing in detail the fatty acid composition of milk fat in 1 to 2 hr. was developed. This method, based on gas chromatographic separation of the fatty acid methyl esters, can be compared with the older, vacuum fractional distillation procedure which requires days to complete an analysis. With the gas chromatographic method it is now possible to explore the composition, structure, and synthesis of milk fat much more extensively than has been possible in the past. The specific differences between summer and winter milk fats were readily made evident by this method. It is envisioned that milk fat may be developed as a much more flexible food raw material, from the standpoint of composition and properties, with the aid of gas chromatography as a research tool. Thus, changes in milk fat composition brought about by feeding and breeding practices may now be easily explored.

STRUCTURE AND SYNTHESIS OF MILK FAT. II. FATTY ACID DISTRIBUTION IN THE TRIGLYCERIDES OF MILK AND OTHER ANIMAL FATS

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The study of lipids is moving to the forefront in biological research because of the apparent role they play in health and disease. Before studying the metabolic action of these substances it is necessary to learn the nature of the compounds. Since the advent of gas chromatography, it has been possible to determine fatty acid composition in a practical period of time. However, in addition to this information, knowledge on lipid structure is essential to describe adequately these metabolites.

The term, triglyceride structure, refers to the relative placement of individual fatty acids on the various positions of the glycerol molecule. It was possible to study this fatty acid placement with the enzyme, pancreatic lipase. This organic catalyst selectively cleaves the fatty acids on the terminal positions of the glycerol, leaving a monoglyceride with the fatty acid in the middle position.

The animal fats studied showed a similar pattern in fatty acid placement except in regard to the positioning of palmitic and oleic acids. In pig fat and

cow milk triglycerides, palmitic acid tended to be concentrated in the middle position; whereas, in the other fats oleic acid was predominantly in this position.

The results for all fats indicated a definite pattern of fatty acid placement, rather than a random distribution on the glycerol molecule. Since blood and milk triglycerides from the same cow differed in structure, it appears that if blood triglycerides contribute to milk fat there must be either a rearrangement of fatty acids on the glycerol molecule or a supplementary synthesis of triglycerides which changes the pattern of fatty acid positioning.

CHEDDAR CHEESE FLAVOR. II. CHANGES IN FLAVOR QUALITY AND RIPENING PRODUCTS OF COMMERCIAL CHEDDAR CHEESE DURING CONTROLLED CURING

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Commercial Cheddar cheese (14 lots from 11 plants) was studied in an attempt to establish relationships between the products of ripening and the desirable cheese flavor. The cheese was analyzed during a 12-mo. storage period for quality, ammonia, hydrogen sulfide, free amino acids, free fatty acids, and acidic and neutral carbonyl compounds.

The cheeses fluctuated in flavor quality during ripening, and the length of time required to reach optimum flavor quality varied in individual lots. For the majority of the chemical products of ripening there was no discernible relationship between the concentrations of the compounds and the flavor quality of the cheese, whether the compounds were considered alone or as families of related compounds. However, the concentrations of ammonia and hydrogen sulfide appeared to have a direct relationship to the flavor of the cheese, with the latter product being particularly consistent in this respect.

Limited evidence indicated that the desirable flavor of Cheddar Cheese might be related to a balance between the concentration of hydrogen sulfide and total free fatty acids. Cheeses with fatty acid–like flavor were relatively low in hydrogen sulfide. Cheeses with sulfide-like flavors were relatively low in free fatty acids; whereas, cheeses with the most desirable flavor, i.e., balanced flavor, had ratios of free fatty acids to hydrogen sulfide midway of the other two groups of cheese. This observation indicates that optimum flavor development in Cheddar Cheese is dependent on the simultaneous release, or formation, at quite specific rates, of both free fatty acids and hydrogen sulfide.

METHANOL-SOLUBLE COMPLEXES OF LACTOSE AND OF OTHER CARBOHYDRATES

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We have found that carbohydrates of low molecular weight are generally highly soluble in concentrated solutions of anhydrous calcium chloride in cold absolute methanol. After some time, crystalline methanol-solvated complexes of

calcium chloride are deposited. Carbohydrates are generally rather insoluble in anhydrous methanol alone. It is suggested that methanolic calcium chloride may serve as a useful solvent for reactions of lactose and other sugars, and may aid in the fractionation of sugar mixtures by extraction or crystallization.

A highly concentrated viscous solution can be prepared by mixing lactose, calcium chloride, and methanol in a molar ratio such as 1:3:24. After the solution is aged, a little of the lactose, less than 3% according to chromatographic analysis, changes to an unidentified carbohydrate which must have a very low or negative optical rotation. The alpha form of lactose exists in higher proportion in methanolic calcium chloride than in water.

From the highly concentrated methanolic solution, heavy crystallization occurs after months or, with seeding and shaking, after several days. Separated by prolonged centrifugal filtration in a closed system, the crystals, which can not be washed without change in composition, are found to consist of lactose (nearly all in the beta form), calcium chloride, and methanol in the molar ratio 1:1:4.

If the concentrated methanolic solution is diluted with methanol, pure lactose crystallizes predominantly in the alpha form. The alpha-beta ratio is almost exactly the reverse of that for lactose at equilibrium in water. Within the range investigated, the logarithm of the solubility of this crystallized lactose in methanol is a linear function of the calcium chloride concentration.

When about 6 *M* of water are added to the concentrated methanolic solution of lactose for each mole of dissolved calcium chloride, the maximum amount of a crystalline complex gradually separates out. These crystals, consisting of alpha-lactose, calcium chloride, and water in the molar ratio 1:1:7 are identical to those deposited, according to Herrington's procedure, from water.

The solubilities of several carbohydrates have been determined qualitatively at 28° C. by the mixing of the carbohydrate with calcium chloride and methanol in the molar ratio of 1:3:24. In general, monoses, bioses, and trioses, as well as their simple derivatives, have been found soluble; only the higher polysaccharides are not soluble. Some of these monoses, notably glucose and fructose, crystallize very rapidly as methanol-soluble calcium chloride complexes. The fructose solution is especially interesting. Soon after it begins to crystallize, it turns into a translucent homogeneous rigid gel which fills the entire volume of the solution.

SPECIFICATIONS FOR THE SPREADABILITY OF BUTTER

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Conventional butter is not easily spreadable at the temperature of the home refrigerator and generally becomes too soft for easy handling at most usual room temperatures. Research to improve butter spreadability is being done in various institutions. However, without the use of objective terms and of a standard instrument it is difficult to appraise the significance of treatments said to improve the spreadability of butter.

By comparing spreadability measurements expressed in grams of resistance with subjective assessments of a panel of 15 persons, it was possible to arrive at specifications for the spreadability of butter.

The experimental work was done in a room where the temperature could be varied from 39 to 72° F. to obtain a wide range of resistance to spreading. The modified Huebner-Thomsen apparatus was used because of its good sensitivity and because in action it simulates the manual spreading of butter or margarine. On each testing day, the 15 panelists were asked to assess independently the handling characteristics of each print of butter or margarine by feeling with the fingers or with a knife, and the spreadability by spreading some of the sample with a table knife on fresh bread.

A straight-line relationship was obtained between the spreadability assessment and the logarithm of resistance to spreading. Taking both handling and spreading into consideration, the specifications found for the spreadability of butter were as follows: Desirable, between 260 and 530 g. of resistance, and Acceptable, between 160 and 890 g. of resistance. The spreadability of margarine was Desirable between 260 and 540 g. of resistance, and Acceptable between 160 and 1,100 g. of resistance. Beyond the limits for Acceptable, the sample was either too soft for handling or too hard for spreading.

WHEY UTILIZATION. IV. AVAILABILITY OF WHEY NITROGEN FOR THE GROWTH OF *SACCHAROMYCES FRAGILIS*

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Whey contains approximately 0.14% nitrogen. The principal nitrogenous components are the heat- and acid-precipitatable lactalbumin and lactoglobulin, whereas the nonprotein nitrogen fraction consists of proteose-peptones, amino acids, and other substances. *Saccharomyces fragilis*, growing in whey without additional N, utilized approximately 25% of the organic nitrogen contents of the whey. In the presence of added $(NH_4)_2SO_4$, the yeast utilized the inorganic nitrogen while simultaneously using the 25% of the organic nitrogen originally present in the whey. Approximately 52% of the organic nitrogen of the whey is precipitable by acid- and heat-treatment. Growth of *S. fragilis* in whey free of the precipitable protein indicated that the available nitrogen in the whey was in the noncoagulable portion. The yeast did not degrade the heat- and acid-precipitable lactalbumin and lactoglobulin, even when growth was limited by the lack of readily available nitrogen.

VARIATION IN RECTAL TEMPERATURE, RESPIRATION RATE, AND PULSE RATE OF CATTLE AS RELATED TO VARIATION IN FOUR ENVIRONMENTAL VARIABLES

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Several investigators have reported the results of studies of physiological responses of cattle to ambient conditions of air temperature and humidity, in many cases closely controlled, in chambers designed for this purpose. These reports constitute valuable contributions to our understanding of the fundamental processes involved in heat regulation in the animal body. The present paper presents the results of experiments designed to study the same phenomena

under natural conditions with environmental variables continuously recorded to permit statistical control rather than the physical control attained in psychrometric chambers. Such studies seem to be logical intermediate steps in the process of applying the fundamental knowledge acquired from control chamber studies to practical problems in breeding and management.

To determine the relative importance of variations in air temperature, wind velocity, solar radiation, and vapor pressure in producing responses of cattle, a series of four 24-hr. studies in the summer of 1953 and five in the summer of 1954 were conducted with Brahman, Jersey-Brahman crossbred, Jersey, and Holstein females. The cattle used were nonlactating females. In 1953 they consisted of four Brahmans, aged 4 to 5 yr.; ten ½ Jersey–½ Brahman, aged 6 to 19 mo.; two ¾ Jersey–¼ Brahman, aged 3 and 10 mo.; ten Jerseys, aged 4 to 18 mo.; and ten Holsteins, aged 6 to 18 mo. In 1954 the number of Jerseys, Holsteins, and halfbreds was the same as in 1953, ten each; but no Brahmans were used and four ¾ Jersey–¼ Brahmans were available. The 1954 age ranges were: ¾ Jersey–¼ Brahman, 6 to 15 mo.; ½ Jersey–½ Brahman, 5 to 19 mo.; Jersey, 6 to 20 mo., and Holstein, 6 to 21 mo.

Thirteen observations each of the rectal temperature, pulse rate, and respiration rate of each animal were recorded during the 24-hr. period, i.e., at the beginning of the test period and at intervals of 2 hr. Continuous records of solar radiation, air temperature, vapor pressure, and wind velocity were obtained. Though the primary object was not a breed comparison, the data conform to the findings of previous studies with respect to the relative heat tolerance of the groups studied. The order of heat tolerance being Brahman, most heat-tolerant, followed by ½ Jersey–½ Brahman, ¾ Jersey–¼ Brahman, Jersey, and Holstein.

The data were studied by a statistical procedure known as multiple regression, using as independent or causative variables the four environmental variables and as dependent variables or effects those measured on the cattle. The results indicate that under conditions similar to those prevailing during these studies, almost as satisfactory explanation of variation in rectal temperature, respiration rate, and pulse rate can be accomplished with air temperature as the only independent variable. The percentages of variation in the measured physiological responses due to all the independent variables did not differ much from those obtained considering air temperature alone.

From a practical standpoint, it may be concluded that air temperature is the most important environmental variable with respect to summer weather stress in cattle. Management procedures to combat summer weather stress should emphasize efforts to provide as low air temperatures as economically feasible. This does not mean, however, that no effort should be devoted to the provision of shade for cattle.

A STUDY OF THE INFLUENCE OF SOLAR RADIATION ON PHYSIOLOGICAL RESPONSES OF DAIRY CATTLE

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During the summers of 1955 and 1956 studies of the influence of weather factors were conducted at College Station, Texas. Eighteen heifers were used

as experimental animals each year. The ages of these animals ranged from 7 to 27 mo. at the time the data were collected. The 1955 studies involved six purebred Jerseys, six ½ Jersey–½ Brahman, and six ¾ Jersey–¼ Brahman. In 1956, six Holstein heifers were used in place of the ½ Jersey–½ Brahman animals, of which there were no animals in the herd under 27 mo. of age when the 1956 studies were conducted. Individual heifers were exposed to sun and shade over test periods of nine alternate days in each year. At the end of a nine-day experimental period each of the 18 heifers had been exposed to direct sunlight for six days and had been kept in shade for three days.

Beginning at 6 A.M. each test day, and hourly until 5 P.M., observations on each animal were made of rectal temperature, pulse rate, and respiration rate. Hourly averages of air temperature, vapor pressure, solar radiation, and wind velocity were computed from continuous records of these weather factors.

Interest in the effects of solar radiation was primary. Statistical analyses of the data showed that solar radiation had a direct effect on rectal temperature in air temperatures that were close to the range in which cattle of European origin are believed to suffer little or no heat stress. In the present study the data were divided into two groups, those collected when air temperature was below 90° F. and those collected when air temperature was 90° or above, 90° F. being the temperature which divided all of the recorded data into two approximately equal groups. In the higher air temperature range changes in solar radiation had little effect on body temperature. Respiration rate, the most consistent physiological response studied, was affected more by solar radiation than by the other weather factors. There were large differences in respiration rate between the three breeding groups studied each year. It could not be demonstrated satisfactorily that variation in pulse rate was consistently influenced by variation in any of the environmental factors studied. Body temperatures and respiration rates of animals in the absence of direct solar radiation were almost as reliable indicators of differences in heat regulatory ability as the same measurements taken on animals exposed to direct solar radiation.

A STUDY OF SOLAR RADIATION AS RELATED TO PHYSIOLOGICAL AND PRODUCTION RESPONSES OF LACTATING HOLSTEIN AND JERSEY COWS

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Twelve lactating cows, six Jerseys and six Holsteins, were used to study the effects of solar radiation on physiological responses and milk production. The cows ranged in age from 4 to 8 yr., and their stages of lactation ranged from 6 to 10 mo. Most of them had been rebred. The study was conducted at College Station, Texas, in August, 1957.

On eight test days six cows, three from each breed, were tied so as to be exposed to direct sunlight, while the remaining six animals were kept in shade. Either one or two days elapsed between test days, and individual cows were alternated between sun and shade locations on successive test days.

Beginning at 6 A.M. and ending at 5 P.M. on each test day, hourly records were taken of each cow's rectal temperature, pulse rate, and respiration rate.

Milk weights for each milking were available for each cow, since the test cows were milked during the regular milking period before the beginning and after the end of each of the eight test days. Data on solar radiation, air temperature, and vapor pressure were available from continuous records routinely taken in the immediate vicinity of the test site. The data were subjected to appropriate statistical analyses.

The body temperatures and respiratory activities of Holsteins and those located in the sun were influenced to a greater degree by the environmental conditions than were the same measurements of the Jerseys and shaded animals.

Air temperature variations were the predominant cause of variations in respiration rate and body temperatures, whereas solar radiation was of considerable importance as a direct cause of increased body temperatures and respiration rates of animals exposed to the sun. For shaded animals increases in solar radiation caused no appreciable direct influence upon these physiological responses. Although slight increases in body temperature and respiration seemed to be associated with increases in vapor pressure, this influence, under the conditions of this test, was slight in comparison with that associated with the other environmental factors. The influence of the environmental factors upon body temperature and respiration rate was considerable, while the influence upon pulse rate responses was so slight that consistent results could not be obtained. The body temperatures and respiratory activities of Holsteins located in the sun were influenced to a greater degree by the environmental conditions than were the same measurements of the Jerseys and shaded animals.

Although exposure to direct solar radiation had considerable effect upon the physiological measurements, it did not cause an appreciable influence upon an animal's milk production. The relationship between the physiological responses and milk production, therefore, may not be as great as is generally assumed.

VARIATIONS IN CERTAIN BLOOD COMPONENTS OF HOLSTEIN AND JERSEY COWS AND THEIR RELATIONSHIP TO DAILY RANGE IN RECTAL TEMPERATURE AND TO MILK AND BUTTERFAT PRODUCTION

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The search for new aids to selection to improve economic traits in farm animals is a continuing one. Since many of these traits are greatly influenced by environment, the true breeding worth of an animal is often badly obscured by fortuitous environmental circumstances prevailing during the animal's lifetime.

Because certain blood components would seem rather logically to be involved in the physiology of animals, attempts have been made to determine the extent of such involvement by determining the levels of these components in the blood and relating these levels to performance with respect to economic traits. Review of the literature brought to light several studies of this kind on various

species of farm animals. Results of published studies have not been in complete agreement, but some seemed indicative enough to warrant undertaking the present investigation.

Winter and summer blood samples were taken from 96 Jersey and 78 Holstein cows. Laboratory determinations of the levels of reduced glutathione and hemoglobin and of red-cell volume were made. The data were analyzed statistically, to determine all relationships of possible interest and meaning, in order to determine the relative importance of various assumed causes of variation in the blood components and in daily range in rectal temperature, another variable of interest observed on cows in milk during the summer.

All of the blood components were repeatable. Age variation seemed to have no influence on variation in the blood components or daily temperature range. Breed and season differences were found, but real sire differences were not observed except in the case of glutathione in the Holstein breed. Stage of lactation and/or stage of gestation appears to have an influence on or be related to hemoglobin and daily temperature range, stage of lactation being the more important in Jerseys and stage of gestation the more important in Holsteins.

Correlations of the blood components with most probable performing ability for milk production, fat production, and fat test were so small that the blood components were concluded not to be useful criteria of selection for these economic traits.

FORAGE INTAKE AND EFFICIENCY OF FEED UTILIZATION IN DAIRY CATTLE[1]

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Research on forage production has resulted in spectacular advances. In view of this changing pattern, attention is now turned to research on differences in the animal itself and to possible improvements in utilization of forage.

Most dairymen recognize considerable differences in the amount of forage consumed by individual cows. But, the significance and use of this characteristic in obtaining greater efficiency of production and increased net profit from a dairy herd are not fully appreciated.

As a factor in economical milk production, forage intake has been studied in 12 Cornell experiments since 1950. A total of 175 Holstein cows were fed various forages to full capacity (ad lib.) for 20 wk., starting at an average of 60 days after calving for their second lactation or beyond. Only one kind of forage was fed, and grain feeding was moderate, with an average of 1 lb. per 4.1 lb. of fat-corrected milk.

For these extensive experiments the average daily dry matter intake from forage was 26.7 lb. per day, supplemented, on the average, with 9.6 lb. of grain. The average daily production of 4% fat-corrected milk was 39.6 lb.; average beginning weight, 1,333 lb.; and average daily change in weight, +0.24 lb. The average efficiency for 20 wk., starting 60 days after calving, was a requirement

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of 56.7 lb. of TDN for maintenance and milk per 100 lb. of 4% fat-corrected milk. The range of the individual cows was from 42.0 to 82.7 lb.

The results showed highly significant differences for forage intake among individual cows; this variable accounted for 75% of the differences. Only 25% of the differences were associated with the three variables of milk production, body weight, and changes in body weight.

The differences in forage intake among cows were very consistent from week to week. The cows had a marked tendency to maintain their rank when changed from a palatable early-harvested to an unpalatable late-harvested forage, regardless of whether it was provided in an all-hay or an all-silage ration. The tendency for cows to maintain their rank in consumption of dry matter was indicated by a high repeatability of the average weekly forage intake and by the fact that three of every four cows in the top half for intake on one kind of forage were also in the top half when changed to an entirely different kind of forage.

The range in cutting dates from June 3 to July 9 showed a progressive change in digestibility from 67.2 to 51.5%, and for consumption a change from 2.72 to 1.95 lb. of hay daily for each 100 lb. of body weight. Early cutting gave an advantage of 39% in hay consumption and 82% for daily digestible dry matter intake, when compared with late-cut forage.

It required the same amount of TDN to produce 100 lb. of 4% milk from early-cut forage as from late-cut forage. However, the cows did not eat enough of the late-cut forage and that consumed did not contain a high concentration of energy. Thus, production was 27% higher for forage harvested before June 15, as compared with late-cut harvested after July 1. Nutrients from silage were consistently superior to those from hay, even though the forage used was harvested at the same time from the same field. The average difference was 5.1 lb. of TDN per 100 lb. of fat-corrected milk; hay required an average of 59.6, and silage 54 5 lb. of TDN.

Efficiency was highly correlated with milk production, and selection for high milk production should cause reasonable improvement in efficiency of feed utilization until some easy method is devised for determining efficiency on the farm. Efficiency from feed was largely independent of body size, because milk production and feed intake increased in approximately the same proportion when there was an increase in size.

The highly significant differences in forage intake among cows reflect individual physiological differences. Some cows apparently eat until they are filled to capacity, whereas others are satisfied when only partially filled. This suggests the possibility of breeding and/or selecting cows with improved appetites to increase forage intake. It suggests also the advantages of ranking cows or heifers into groups based on forage intake, after a preliminary period before they are assigned at random for a feeding experiment.

EFFECT OF CONDITION OF ANIMAL ON THE REQUIREMENTS OF ENERGY FOR MAINTENANCE OF THE BOVINE

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Farm animals are fed to maintain body functions such as heart and lung

action and to stimulate production such as growth or milk secretion. Feed nutrients needed by animals are listed according to needs for maintenance and production in most feeding standards used by dairy scientists and feeders. Maintenance requirements for energy are allotted according to weight of animal to approximately the three-quarter power. That is, as weight increases energy requirements also increase, but only three-quarters as fast as weight increments. Experiments have suggested that this relationship does not hold if weight increments are made up mainly of fat.

From the work reported, it becomes clear that in cases in which weight increments are largely due to increased condition, the requirements for energy increase per unit weight rather than decrease, as in the normal situation. There is practical significance to this situation.

This higher requirement of high-condition animals for energy to maintain their weight may be an explanation of why high-condition heifers give no more milk than low-condition heifers that are both fed according to standards after they come into milk. It is the most probable explanation of why highly fed animals convert feed to milk less efficiently from a nutrient standpoint than animals receiving somewhat less feed.

Feeding thyroprotein to dairy animals stimulates metabolic activity as in other animals. Such stimulation might increase efficiency of feed conversion, might not affect it, or could affect it adversely. In these trials, in which it was given to bulls in 15-g. amounts per day, it had the very definite effect of causing gains to be made more slowly and less efficiently (pound gain/unit feed), and had the effect of causing maintenance requirements to be increased per unit weight.

At present there is no way to estimate fatness of animals in order that effects of condition on requirements can be estimated accurately. The findings throw doubt on the expediency of calculating maintenance requirements of energy on a weight to the three-quarter power basis. A good deal of the weight differences seen between animals of a breed in a herd might well be due to fat. It might be well to revert to the practice of allotting feed for maintenance in direct proportion to weight.

LACTATION STUDIES. II. COMPARISON OF TECHNIQUES TO MEASURE TRUE MILK AND FAT SECRETION RATES IN DAIRY CATTLE AS APPLIED TO BETWEEN-MILKING INTERVALS OF VARIOUS LENGTHS OF TIME

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Work in years past has been concerned with the problem of how milk and butterfat secretion rates are affected by accumulation of milk in the udder of the cow. It was learned that when heavy-producing cows were milked more

often, more milk and fat were produced per unit time. In recent years it has been observed when cows are milked on a skewed-hour schedule, e.g., a 10-14 hr. schedule, that little difference in daily or yearly production resulted compared to an equal-hour schedule. However, when one looks at the apparent secretion rates of milk and fat on a within milking interval basis it is seen that production during the shortest interval in the day is highest in both milk and fat.

An explanation was forthcoming when it was found that part of the complementary milk (that removable by use of oxytocin) present after and produced during the long interval was released after the short interval. Certain studies then purported that when cows were milked on a skewed-hour schedule differences in complementary milk explained all the differences seen in the apparent secretion rates between the two different intervals. It was implied from this work that milk secretion rate was not affected by accumulation of milk in the udder until an elapsed time of about 20 hr.

The work reported here was initiated to compare methods of measuring true secretion rates of milk and fat, using oxytocin as contrasted to the apparent rate, and then to apply the most reliable method to secretion rate studies.

The system whereby oxytocin was used at consecutive milkings proved most reliable.

In our study, in which true intramilking interval secretion rates were studied on three different milking schedules, it was found that secretion rates were depressed as the interval was lengthened. There was no difference in the secretion rates between two 12-hr. intervals; they showed a difference which was not significant when an 11- and 13-hr. schedule was used, and became statistically significant on the 10-14-hr. schedule.

The conclusion to be drawn from this work is that the difference noted in apparent secretion rates seen when cows are milked on a skewed-hour milking schedule are partly due to a carry-over of complementary milk, but are also partly due to the fact that milk secretion rates are depressed as the milking interval is increased.

CHANGES IN THE TISSUE AND VOLUME OF THE STOMACHS OF CALVES FOLLOWING THE REMOVAL OF DRY FEED OR CONSUMPTION OF INERT BULK

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The marked increase which takes place in the capacity of the reticulo-rumen as the young ruminant increases its consumption of dry feed, and the influence this organ has over feed consumption and digestion, have prompted frequent descriptions of the anatomical changes taking place during rumen development. Research into the causes of this development has been relatively recent, however, and has shown that at least three changes take place, viz., development of capacity, mucosa, and musculature. Of these, the mucosa is the most studied and has been shown to grow finger-like papillae in response to the absorption of propionic and butyric acids. The permanence of this development had not been tested and a more complete description of the nature of the growth of

the stomach muscle as well as mucosa, particularly in the rumen, seemed to be needed. A fuller understanding of the process of rumen development will help in evaluating future observations in this area, and eventually lead to a sounder basis for calf-feeding recommendations.

In the present experiment high-roughage (nine parts hay to one part grain) and high-concentrate calves (1:9) were fed these diets for 16 wk. At 16 wk. of age four animals were slaughtered and observations were made of the stomach development in this group. These formed a base line from which to evaluate the eight other calves, four of which were reversed to a milk diet and housed off bedding for 20 wk., and four of which were retained on their normal diets for a like time. Two more calves were raised on milk alone and two on milk while being allowed to consume bedding (shavings). Rumen papillae disappeared in calves reversed to milk. Their rumens were collapsed and had less fill, but maintained the same potential (water-filled) volume as was present in the dry feed calves, which were used as a base line. This indicated a stoppage in rumen growth, but no shrinkage of volume. The omasum likewise did not continue to grow, but the abomasum continued to grow at the same rate as the body. Rates of muscular and mucosal retrogression were different, the nitrogen composition of these two tissues was different, and extensive muscular growth took place in calves consuming shavings, even though there was no mucosal development. These three differences between the two tissues indicate that rumen muscle and mucosa develop independently. Rumen papillae length was greater in the faster-gaining high-concentrate calves, probably due to the greater amount of volatile fatty acids produced and absorbed on these diets.

Since an extensive development of both rumen mucosa and muscle is always seen in an animal with an actively functioning rumen, and since these two tissues develop independently, a calf-feeding and management scheme which stimulates the growth of both tissue types as well as rumen capacity must be used if complete development of rumen structure is to be accomplished. The retrogression of the rumen papillae in the absence of a rumen fermentation indicates that these structures are not static and will change with varying conditions. Correct evaluation of the significance of papillary growth can, therefore, be made only if the fermentable energy intake of the animal is known.

UREA UTILIZATION BY YOUNG DAIRY CALVES AS AFFECTED BY CHLORTETRACYCLINE SUPPLEMENTATION

L. D. BROWN, D. R. JACOBSON, J. P. EVERETT, JR., D. M. SEATH, AND J. W. RUST
Department of Dairy Science
University of Kentucky, Lexington

It has been well established that calves two to four days of age raised to 3 mo. of age on the limited milk hay starter system make normal gains whenever the starter contains 12 to 16% plant crude protein. This is also true when urea is substituted for one-third of the crude protein equivalent in the starter.

It has often been observed that certain antibiotics give rise to an increased growth rate in young calves. The increased growth rate has practically always been associated with increased feed intake. The results of this paper support all of these observations.

What is not well understood is the mode of action of the antibiotics and whether they would give the same growth stimulus in a ration containing urea.

The data in this paper give clear indication that the mode of action of the antibiotic chlortetracycline is not that of sparing dietary protein, as has been proposed as a possibility. In fact, the opposite trend was observed, i.e., that poorer growth was obtained by the calves that received the chlortetracycline in the low protein group and greater growth in the calves that received the chlortetracycline in the highest protein groups.

The efficiency of utilization of feed was examined in this study by calculating the TDN above maintenance required per pound of gain and by the analysis of covariance where starter consumption was adjusted. In either case there was a significant difference in favor of the calves that received the antibiotic. This means the chlortetracycline caused an increase in gain beyond that attributable to the increased intake.

The effect of chlortetracycline supplementation on calf performance and ration utilization where dietary urea was included was much the same as that observed previously where native protein was employed.

SURVIVAL OF BOVINE SPERMATOZOA STORED AT 5 AND 25° C. IN EXTENDERS CONTAINING VARYING LEVELS OF EGG YOLK, GLUCOSE, GLYCINE, GLYCEROL, CITRATE, AND OTHER SALTS

R. H. FOOTE AND R. W. BRATTON
Department of Animal Husbandry, Cornell University, Ithaca, New York

Considerable progress has been made in defining the environmental conditions favoring maximum survival of unfrozen spermatozoa. However, the fact that simple changes in the medium or storage conditions often result in longer survival than was previously possible to achieve indicates that the upper limit has not been achieved. If survival and fertility of spermatozoa during storage could be prolonged easily, it would increase the possibilities of livestock improvement through artificial insemination, and reduce the cost of such a program.

Studies were undertaken to compare the effects of various salts, and other ingredients incorporated in different concentrations in semen extenders, the effect of storage temperature, and the effect of storing in corked versus sealed containers on the survival of spermatozoa. It was found that lower concentrations of ingredients than are normally considered to be isotonic with spermatozoa favored sperm survival, and that partial replacement of citrate buffer with glucose and glycine was desirable. Spermatozoa stored at 5° C. survived longer than those stored at 25° C., although the addition of sulfanilamide partially overcame the detrimental effect of higher temperature. Survival of spermatozoa was similar in corked tubes and sealed ampules. Under the optimal conditions in these experiments (storage in either ampules or tubes at 5° C. in an extender containing 20% egg yolk, 1.16% sodium citrate dihydrate, 1.0% glucose, 0.75% glycine, 0.3% sulfanilamide, and antibiotics, or in a slightly more complicated extender) the percentage of motile spermatozoa was main-

tained at a higher level during storage than has generally been reported, suggesting that fertility of bull semen stored for several days might also be improved.

FERTILITY OF BULL SEMEN STORED UP TO FOUR DAYS AT 5° C. IN 20% EGG YOLK EXTENDERS

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AND

H. O. DUNN
New York Artificial Breeders' Cooperative, Inc., Ithaca, New York

More than six million dairy cows, and an increasing number of beef cows, are being inseminated artificially each year. Industry-wide increases in fertility as small as 1% would be of considerable economic value. One possible way of bringing this about is through the use of improved extenders capable of maintaining a higher level of fertility over a storage period of several days.

Three field trials comparing a 20% yolk-citrate-sulfanilamide-glucose-glycine extender (CU-16), and a similar extender containing in addition sodium bicarbonate, potassium chloride, and citric acid (CUE) were compared with a 50% yolk-citrate-sulfanilamide control extender. All extenders contained penicillin and dihydrostreptomycin. Semen was used over a four-day storage period at 5° C. More than 6,000 first inseminations per extender were obtained, and the 60- to 90-day nonreturn percentages were 73.4 for the control, 76.6 for the CUE, and 76.0 for the CU-16. The New York Artificial Breeders' Cooperative, Inc. adopted the CUE for routine use, and the fertility increased simultaneously two percentage units. In routine operation 290,053 first services have averaged 74.9% 60- to 90-day nonreturns. For an organization breeding 500,000 cows this improvement represents 10,000 more cows conceiving to first service, or one more cow in each 50-cow dairy.

FREQUENCY OF EJACULATION IN THE BOVINE

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University of Minnesota, St. Paul

As the use of frozen semen increases, the advantages of an increased volume of semen in a given period of time become obvious. This larger volume would allow a breeding association to freeze larger quantities of semen at one time, thereby reducing the number of collection periods. Similarly, if a bull can be collected more frequently than was previously believed, the total number of bulls needed in an organization can be reduced.

It was the purpose of this study to determine if a large number of successive ejaculates could be collected in a single collection period without impairing the semen quality or the libido of the bull.

In a second study, bulls were collected at much higher frequencies of collection periods to determine if such frequencies were detrimental to semen quality, quantity, or bull libido.

The results of the first study indicate that as many as seven ejaculates can

be collected once a week for a period of 1 yr. without causing any decrease in semen quality or libido which would prove detrimental in a breeding organization. The volume of semen produced per week is increased over the control due to the increased number of ejaculates. Although the higher number of ejaculates per collection period resulted in lower motility ratings, all semen was of acceptable motility for breeding purposes.

It is concluded that bulls can be collected more frequently than was previously believed practical, and by this technique and the collection of greater numbers of ejaculates per collection period, the volume of semen collected in any given period of time can be greatly increased.

CORRELATION OF CERTAIN BLOOD CHARACTERS IN THE HEIFER WITH HER MILK-PRODUCING ABILITY

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Ways of predicting future production of young dairy heifers have been sought by many investigators. Studies of dairy conformation, mammary gland development in the calf, and many physiological studies have been made with this purpose in mind.

The purpose of this study was to determine whether certain blood constituent levels in the young heifer are related to her milk-producing ability later in life. The blood constituents studied were erythrocyte count, hemoglobin, and hematocrit values. Determinations of these constituents were made monthly on heifer calves from 3 to 10 mo. of age. Thus, eight separate determinations were made for each calf. Because the blood characters studied are influenced by a number of factors, it was deemed necessary to obtain a number of estimates on the blood of a particular heifer, to get a reliable estimate of the individual's blood picture. The average of these eight determinations was correlated with the heifer's 3.5% F.C.M. production later in her life.

This report is of a preliminary nature because the number of heifers on which blood character level and milk production records are available are limited (53 Holstein and 20 Jersey), and all are from one herd; nevertheless, it appears that the magnitude of the red cell count in the heifer calf is rather consistently related to her future milk-producing ability—the higher the mean red cell count in the calf 3 to 10 mo. of age, the higher the milk production. Expressed statistically, the relationship is $r = +.5$.

AN EMULSIFIED SOYBEAN OIL FOR BLOAT THERAPY

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Department of Animal Husbandry, Iowa State University, Ames

Frothing of rumen contents now is accepted quite generally as the immediate cause of pasture bloat. Thus, the need for effective defoaming agents for treatment of acute bloat is obvious. The current report discusses an emulsified soybean oil product which was found to be highly effective in treating bloated cattle and sheep. This product contains no expensive, irritating, or toxic ingredients, being composed of crude soybean oil, ethanol, casein, sodium car-

bonate, and water. It is water-dispersible, which facilitates distribution in the rumen.

The emulsified product was administered to sheep by drenching. In cattle, the usual method of administration was by stomach tube (about 8 ft. in length and at least ¾ in. inside diameter). The stomach tube probably is the method of choice, at least in cattle, since when used properly it sometimes permits release of some free gas immediately upon insertion and usually permits release of larger quantities of gas promptly following administration of the emulsified oil.

OUR ASSOCIATION

A CLASSIFICATION OF MEMBERSHIP IN THE AMERICAN DAIRY SCIENCE ASSOCIATION

H. F. JUDKINS, Secretary-Treasurer
32 Ridgeway Circle, White Plains, New York

In this fast-moving world, it is well to pause occasionally and take stock of our Association, to see how the membership is changing.

Article III, Section 1, of the Constitution reads, "Any person shall be eligible to membership who has had college training in dairying or who is in a position of responsibility that requires a technical knowledge of dairy science." This allows for considerable latitude, since most any job in the dairy field except that of a laborer requires some technical knowledge.

MEMBERSHIP HISTORY

A study of 50 yr. of Association history, prepared by Historian Dr. G. M. Trout for the 50th Anniversary in 1956, reveals the following historical background of the Association.

1906—Association founded at University of Illinois. The 18 charter members were teachers and investigators. Name, National Association of Dairy Instructors and Investigators.

1907-08—Name changed to Official Dairy Instructors Association.

1916—Name changed to American Dairy Science Association.

1917—JOURNAL OF DAIRY SCIENCE established.

MEMBERSHIP RECORD

1906—8 charter members
1915—144 members
1925—261 members
1935—735 members
1945—1,407 members
1955—1,775 members
1959—2,587 members

Membership growth was not phenomenal until Professor R. B. Stoltz of Ohio State University became secretary-treasurer in 1936, a position he held until 1948. Professor Stoltz took great interest in the growth of the Association and organized a campaign with a member in each state responsible for membership. This is essentially the plan that the Membership Committee has followed for the past 3 or 4 yr. The results in increased numbers are clearly evident in the figures cited, but they do not reflect changes in the composition of the membership. All of the charter members were teachers and research workers and this continued to be the main composition of the membership for about 30 yr. It is evident that if every teacher and research worker in all universities and in the U. S. Department of Agriculture belonged to the Association, and most of them do, this group would constitute less than half of the present membership. It is obvious that the membership must have become more diversified.

PRESENT MEMBERSHIP CLASSIFICATION

To get an idea of the extent of this diversification, the dues notice for 1960 asked the following questions:

a. Name of present employer
b. Type of business
c. Type of your position
d. Field of principal interest (Association section)

Several failed to answer these questions, making a special letter of inquiry necessary. By April 15, information was obtained for 2,278 members out of a total of 2,472. It is felt certain that the 200 missing reports would not materially change the membership classification on a percentage basis. Table 1 summarizes the results.

It will be noted that more than half the membership is interested in manufacturing. Only 43.1% of the total are engaged in teach-

TABLE 1
Member interest and those engaged in teaching and/or research

Interest	No. of members	Per cent of total	No. in teaching and research	Per cent in teaching and research
Manufacturing	1,225	54.2	417	34.0
Production	857	37.2	561	65.5
Extension	196	8.6	5	2.5
Total	2,278	100.0	983	43.1

TABLE 2
Members from universities and USDA as compared to all others

Interest	No. from universities or USDA	Per cent from universities or USDA	No. others	Per cent others
Manufacturing	281	12.3	944	41.5
Production	472	20.7	385	16.8
Extension	122	5.4	74	3.3
Total	875	38.4	1,403	61.6

ing and research. Of those engaged in teaching and research, 65.5% are in the production field.

Table 2 classifies the interest of members from universities and the USDA as compared to all other members. Since virtually all of those from universities and the USDA are engaged in teaching or research or extension, the difference between 43.1% in Table 1 and 38.4% in Table 2 of 4.7% represents the other members engaged in research. Table 2 shows that there are nearly twice as many teachers and researchers interested in production as there are in manufacturing. On the other hand, industry and other members are more interested in manufacturing, 41.5 to 16.8%.

The figures in Tables 1 and 2 certainly show changes in the composition of membership over the years; the greatest changes undoubtedly occurred during the last 5 to 10 yr.

The work or business in which members are engaged is shown in Table 3.

One is impressed by the variety of businesses involved. Particularly noteworthy is the increased interest in dairy manufacturing and in the manufacture and sale of equipment and supplies. In the production field, the fast-growing artificial insemination business has proved a fertile field for new members and the increase in membership of dairy farm operators and feed manufacturers has been signfiicant.

TABLE 3
Membership classification by type of work and by Association sections
(Cases where five or more are in same type of work)

	Sections					
	Manufacturing		Production		Extension	
Type of work	Total	No. in research	Total	No. in research	Total	No. in research
Breed association			9	1	5	
Breeding association			68	5	5	
College extension					120	
College teaching and research	248	248	425	425	13	
Consultant	20		6		1	
Co-op. marketing and purchasing	8		19	6	4	1
County agent or assistant			5		16	
Dairy equipment and supplies	148	39	43	22	4	2
Dairy farming	1		69			
Dairy manufacturing	582	88	8	1	2	1
Feed distribution and farm supply	1	1	14	4		
Feed manufacturing	17		74	37	1	
Food distribution	10		1			
Military	10	4	1			
Private laboratory	37	6	3	3		
Publishing	5		7		1	
Public health	27		7			
Retired	17		4			
State department of agriculture	18	6	23	7	7	1
Trade association	31		8	1	3	
USDA	33	24	47	43	2	
Veterinarian			12			
Miscellaneous[a]	12	1	14	6	8	
	1,225	417	857	561	196	5

[a] Types of work with less than five members include American Embassy, Civil Engineer, Dairy Council, Fulbright, I.C.A., Life and Hospital Insurance, Sausage Manufacture, British Embassy, D.H.I.A. Computing Service, Seed Company, United Nations, F.A.O., Electroplating, Farm Real Estate, Meat Packing, Poultry Breeding, and Rockefeller Foundation.

It is of interest to note the variety of positions held by our members in some of the large group classifications and, particularly, the large number of executives and business owners represented.

Dairy manufacturing	No.
Production manager or plant superintendent	151
Executives representing nearly all phases of the business (40 company presidents)	74
Dairy equipment and supply, manufacture and sales	
Sales manager or engineer	47
Executives	31
Technology and service	26
Engineer	8
Production manager	7
Dairy farming	
Owner	33
Manager	30
Herdsman	5
Breeding association	
Executive	39
Co-operative selling or purchasing	
Executive	18
Feed manufacture	
Executive	21
Sales	15
State department of agriculture	
Executive	12
Milk inspector	4
Veterinarian	4
United States Department of Agriculture	
Foreign aid	9
Marketing	8
Inspection	3
Executive	2
Trade association	
Executive	28
Field representative	2
Private laboratory or institute	
Owner	16
Food technologist	3

FOREIGN MEMBERSHIP

Each year the Association is becoming more international. A considerable number of new members have come through cooperation with the International Cooperation Administration. Table 4 shows the distribution of foreign membership as of April, 1960.

Each year the Association loses about as many members as it gains. This year, as of May, 57 members cancelled their membership and 266 failed to renew. The Association lost 313 members and gained 232 new ones. The length of membership for those lost is shown in Table 5.

TABLE 4

Distribution of foreign membership

Country	No. of members
Mexico and Central America	13
South America	17
Australia	5
New Zealand	2
Philippines	2
Cambodia	1
West Indies	5
Europe	27
Asia[a]	44
Africa	3
Canada	91
Total	210, or 8% of membership

[a] Japan and India have 31 members.

TABLE 5

Period of membership for members lost

Period	No. lost	Per cent
(yr.)		
1-2	171	54.6
3-5	91	29.1
Over 5	51	16.3

More than half of the members leave after 1 or 2 yr. The more common reason is dissatisfaction with benefits derived from membership, notably inability to understand many of the JOURNAL research papers or lack of time to read them.

Statistics were available on the type of work engaged in by some members lost:

Dairy manufacturing	48
Teaching and research	33
Manufacturing and sale of dairy equipment and supplies	13
Dairy farming	11
State department of agriculture	9
Extension work	7
County agents	7
Feed manufacturers	6
Milk producer associations	6

For the most part, these figures show that members lost are users rather than producers of research.

A.D.S.A. MEMBERSHIP AS RELATED TO SERVICES RENDERED BY THE ASSOCIATION

It is obvious from a study of the membership that there are more who can be classed as research users than as research producers. No

claims are made as to whether or not this is as it should be, but this fact must be faced.

President C. A. Elvehjem[1] of the University of Wisconsin, speaking before the last meeting of the National Dairy Council said, "The practical value of any scientific research depends primarily on how well the general public understands the results of that research." In complimenting the Dairy Council health education program for its success in helping to translate nutrition science findings into practical health programs for all people, Elvehjem also said: "The interpreter is the key. The scientist advances knowledge. The interpreter advances progress."

The Association's Executive Board, meeting in June, 1959, recognized the fact that if the Association is to exist and expand along present lines something should be done to benefit the research users or information seekers in the membership. The President was instructed to appoint a Committee on Publication Policy. This committee met in Miami, Florida, in October, 1959, and after considering various avenues of approach decided to recommend the inclusion of Interpretive Summaries of research papers in the JOURNAL. The JOURNAL Management Committee and Executive Board, by majority vote, approved the plan and this new section was added to the JOURNAL on a trial basis, starting with the January, 1960, issue.

The Executive Board meeting in Logan, Utah, at the 55th Annual Meeting of the Association, voted to remove the Interpretive Summaries from the JOURNAL at the end of this year.[2] Starting with January, 1961, a digest of the JOURNAL papers appearing during the first three months will be prepared and sent to the members of the Association. These digests will aim to make the original papers more understandable for some, as well as time-savers for many. It is hoped that they may be well received, particularly by the members who are not interested in research. If this proves to be true, then the membership will become more stable and new members will be encouraged.

At the 1959 Annual Meeting, university and USDA registrants numbered 751, as against 305 from industry. At this year's meeting, two very interesting programs were held for extension workers and others in the dairy business. This type of program is being continued for this part of the membership and as it becomes better publicized it should attract more interested people to the Annual Meeting.

[1] J. Dairy Sci., 43: 429. 1960.

[2] The Journal Management Committee in its meeting at Cornell University, Ithaca, New York, in April, 1960, recommended this action.

CALL FOR PAPERS FOR THE 1961 ANNUAL MEETING OF THE AMERICAN DAIRY SCIENCE ASSOCIATION[1]

G. W. TRIMBERGER
Chairman, Program Committee, A.D.S.A.
Department of Animal Husbandry, Cornell University, Ithaca, New York

The 56th Annual Meeting of the American Dairy Science Association will be held June 12-14 at the University of Wisconsin, Madison. Members who wish to present papers must submit titles and abstracts not later than March 1. This deadline must be met to permit publication of titles with the complete program in the May issue and abstracts in the June issue of the JOURNAL. Last year all but four abstracts arrived on time. If a large number of papers are suggested for presentation, those that arrive late may be assigned to be read by title or rejected entirely.

Abstracts must contain no more than 200 words by actual count. Abstracts longer than 200 words cannot be accepted and will be returned to the author(s) by the Committee. An original (on bond paper) and three copies of each abstract must be typed double-spaced. The original and one copy should be mailed to the Chairman, another copy to the Vice-Chairman, and the fourth to the Secretary of the Section before which the paper is to be presented. Eventually, the original copy will be sent to the Editor for publication in the JOURNAL.

In the preparation of abstracts, the style and abbreviations of the JOURNAL OF DAIRY SCIENCE must be used. Please refer to abstracts in the June, 1960, issue of the JOURNAL for guidance. Only initials of authors should be used, except in unusual cases where it may be necessary to use the complete name. The title of the abstract should indicate clearly the nature of the research. It should not be repeated again in the text. The abstract should include, insofar as possible, the design and major results of the investigation. Only completed research should be reported. Brief, essential statistics will make the data more meaningful.

All members of the Association are entitled to present papers. Participation by members

[1] The procedures for preparing abstracts will apply also to abstracts submitted for the Eastern, Southern and Western Divisional Meetings of the American Dairy Science Association. Editor-in-Chief.

of the industry and by senior members of the Association is particularly encouraged. Attention is called to the Dairy Manufacturing Extension Section program, and members are urged to participate. This is a subsection of the Manufacturing Section for 1961. The competitive presentation of papers by graduate students, initiated in the Production Section in 1959 and in the Manufacturing Section in 1960, will be continued in both sections.

Papers submitted for the Annual Meeting should be confined to research that has not been reported previously. Abstracts of research articles accepted for publication by a scientific journal before the annual meeting are not acceptable, because this constitutes a duplication. The Program Committee favors the general policy that an individual present only one paper and that his name appear as author on no more than two. The Program Committee, together with the Association membership, wishes to stimulate vigorous, sound research and realizes that the above restrictions may penalize some members engaged in full-time research. Therefore, the Committee has liberalized this policy to the extent that a member can exceed these limits, but only if he or his department rates the abstracts in order of preference for oral presentation. If the total number of papers submitted by the membership is too great to include in the program, the Committee will assign some papers to be read by title only. In this event, consideration will be given to quality of abstract and research reported therein, number of abstracts per author and department, and importance of the research reported.

The Program Committee encourages continued improvement in quality of papers and in oral presentation. Careful design of experiments and proper interpretation of results are necessary prerequisites. Strict compliance with the instructions for preparation of abstracts will simplify the task of the Program Committee, and will improve the program. Careful editing of abstracts before submission is essential. In the past too many abstracts have been lacking in this respect.

All abstracts must be submitted on regular 8½ by 11-in. paper. The following form, with no capitals for the title, is correct and is in accordance with the form used in 1960:

> Utilization of carbohydrates posterior to the rumen-reticulum of the bovine. J. T. Huber and N. L. Jacobson, Iowa State University.

If the author lists an address for an experiment station other than the University, or a USDA research branch, or a commercial company, then the complete address should be provided, as presented in the following example from the 1960 program:

> A study of dye reduction methods as platform tests for the detection of antibiotics. Burdet Heinemann, Producers Creamery Co., Springfield, Missouri.

Preparation of mimeographed copies of pertinent data is desirable. At least 250 copies should be available for distribution. This can be supplemented by slides for projection on screens, provided the author can adhere to the assigned time of 12 to 14 min. for presentation of each paper.

Names and addresses of officers of sections to whom titles and abstracts should be sent are:

EXTENSION SECTION

Chairman: M. E. Senger, Department of Animal Industry, North Carolina State College, Raleigh.
Vice-Chairman: W. R. Van Sant, Department of Dairy Science, University of Arizona, Tucson.
Secretary: C. D. McGrew, Department of Dairy Science, Ohio State University, Columbus, 10.

PRODUCTION SECTION

Chairman: S. D. Musgrave, Department of Dairying, Oklahoma State University, Stillwater.
Vice-Chairman: J. C. Thompson, Ralston Purina Co., St. Louis, Missouri.
Secretary: L. H. Schultz, Department of Dairy Husbandry, University of Wisconsin, Madison.

MANUFACTURING SECTION

Chairman: Burdet Heinemann, Producers Creamery Co., Springfield, Missouri.
Vice-Chairman: M. L. Speck, Department of Animal Industry, North Carolina State College, Raleigh.
Secretary: D. M. Graham, Pet Milk Co., Research and Development Center, Greenville, Illinois.

(The Dairy Manufacturing Extension Section is a temporary subsection in the Manufacturing Section. R. B. Redfern, Dairy Extension, North Carolina State College, Raleigh, is chairman and W. S. Arbuckle, Department of Dairy Husbandry, University of Maryland, College Park, is secretary. All abstracts for papers in the Extension Section should be submitted through the regular channels of the Manufacturing Section, but should be identified for presentation at Extension Section meeting and a courtesy copy be sent to R. B. Redfern.)

BOOK REVIEWS

Physiology of Lactation. Vearl R. Smith. Iowa State University Press, Ames, Iowa. 291 pp. 1959. $4.95.

This is the fifth edition of the well known book in the field of milk secretion. The previous editions were entitled Secretion of Milk, by Espe and later by Espe and Smith. The book is concisely written and covers the areas of the anatomy of the mammary gland, the physiology and biochemistry of milk secretion, as well as the environmental and physiological factors which affect the growth and development of the mammary gland and the formation and ejection of milk. It has been revised and brought up to date, with the sections on the biochemistry of milk formation and on the environmental factors affecting lactation being extensively revised.

The author has written basically on the cow, but mammary gland development and lactation in other animals are discussed where they illustrate a point or where research applicable to lactation in general was done on some other species.

The book is extensive in that it covers all phases of lactation and the factors which affect it, and it is written so that it is an excellent text for students at the undergraduate level and also a basis for graduate study.

R. L. Hays
University of Illinois
Urbana

The Merck Index. Paul G. Stecher, editor. 7th ed. 1641 pages; Merck and Co., Inc. Rahway, N. J. 1960. Price $12.00.

The new enlarged edition of this valuable reference book contains nearly 10,000 descriptions of individual substances, more than 3,300 structural formulas, and 30,000 names of chemicals and drugs alphabetically arranged and cross-indexed.

The cross listing is an outstanding feature of the book. It enables the user to locate a particular chemical description by page number regardless of whether he knows only the generic name, brand name or systematic chemical name for the substance.

Another outstanding feature of this book is the section which lists more than 400 organic "Name" reactions with original and review references, together with a description and structural representation of each reaction. The periodic table is arranged in accordance with the latest concepts of nuclear science. Nearly 300 pages are devoted to chromatographic absorbents, Russian alphabet, biological units, coal-tar colors, thermometric equivalents, antifreeze mixtures, refractive index of liquids, saturated solutions, radioactive isotopes, percentage solution tables, isotonic solutions and atomic weights and their multiples and logs.

The preferred chemical name, common or popular names, generic and trade names for each entry are included. Properties and sources of a substance, methods of preparation, literature references and indications for use for each entry are also given.

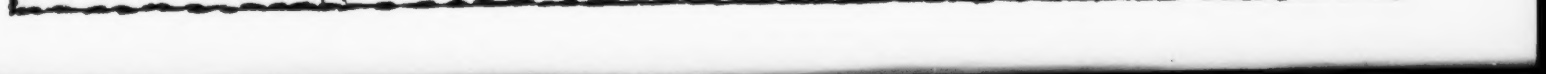
The extensive information about each entry makes this a book that is of much value to anyone requiring such a reference.

R. P. Link
University of Illinois
Urbana, Ill.

Principios de Produccion Lechera. Cesar Fernandez-Quintilla. Salvat Editores, S. A. Barcelona and Madrid. 486 pp., 158 illus. 1960.

This book is a translation in Spanish of Principles of Milk Production by William B. Nevens, eminent Emeritus Professor of Dairy Science at the University of Illinois. Published by McGraw-Hill Company, New York. The translation has been carried out with exacting detail. The translator has included an additional chapter on species of forage of greatest interest in Spain.

E. O. Herreid
University of Illinois, Urbana



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